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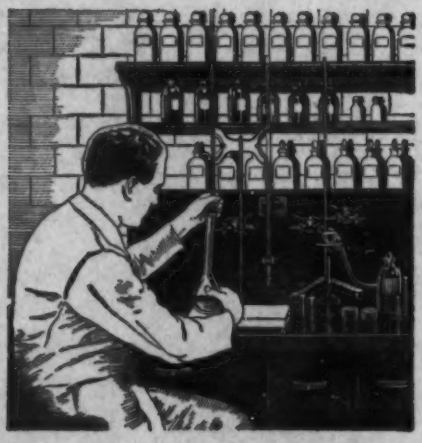

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The Journal of Laboratory and Clinical Medicine

VOL. 20 SEPTEMBER, 1935 NO. 12

CLINICAL AND EXPERIMENTAL

SURGICAL MAGGOTS*

A STUDY OF THEIR FUNCTIONS IN WOUND HEALING

FREDERICK C. MESSER, B.S., AND ROBERT H. MCCLELLAN, M.D., PITTSBURGH, PA.

INTRODUCTION

THE value of blowfly larvae in the treatment of chronic osteomyelitis and of other infected wounds is becoming more widely appreciated, and surgeons are using them with increasingly greater confidence.[1,2] Little has been learned, however, as to the specific ways in which they influence wound healing, or the means by which they are able to exert such influence. Those who have had opportunity of observing larval therapy agree in general that when placed in infected wounds, maggots decrease the bacterial population, remove necrotic tissue, and stimulate healing. There is unfortunately little exact data to prove causal connection between the presence of maggots and any of the above effects. Some objective evidence exists to support the first, but the latter two are founded on clinical observation. In the last analysis, they must remain a matter of opinion until controlled experimental data support them.

Since observations on the healing of clinical, and even of experimental lesions, are difficult to control, it would seem more promising to study certain phases of the physiology of blowfly maggots, in order to demonstrate in what manner, and to what degree they could produce their alleged effects. We have chosen this latter mode of attack in the work reported in this paper.

*From the John C. Oliver Memorial Research Foundation, Ste Margaret Memorial Hospital.

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THE SOURCE OF pH CHANGES IN OSTEOMYELITIS WOUNDS TREATED WITH BLOWFLY MAGGOTS

Baer[1] made the striking observation that wounds treated with maggots became sufficiently alkaline to turn red litmus blue. Litmus is completely transformed to its blue alkaline modification at about pH 8.3,[2] but has a bluish tinge at lower alkalinities. Consequently, this indicator gives only a vague idea of the actual hydrogen ion concentration. In order to study more precisely the reaction of wounds healing in the presence of maggots, the hydrogen ion concentration of a number of such wounds, and of wounds of other types treated only with neutral wet dressings, was followed electrometrically over extended periods of time.

Our earlier measurements were made upon exudate from the wound cavity aspirated into an oiled tuberculin syringe without a needle, and transferred directly to the quinhydrone electrode apparatus of Geselle.[4] Later measurements were made by means of contact electrodes projecting into the wound, permitting readings when the quantity of exudate was too slight to be aspirated. Both systems were checked against Clark and Lub's buffers at three different reactions, and found to agree within 0.2 pH.

Fig. 2 shows fluctuations in hydrogen ion concentration encountered in five operative wounds in chronic osteomyelitis treated with maggots, and in two open wounds of other natures dressed only with wet packs of sterile physiologic salt solution. From the osteomyelitis curves two observations may be made; first, that in all but one case the hydrogen ion concentration fluctuated widely; and second, that in every case, it at some time reached a reaction more alkaline than pH 7.4. In the two cases not treated with maggots, the fluctuations were not so marked, nor did they ever extend above pH 7.4. We have followed the hydrogen ion concentration in eight cases of chronic osteomyelitis and have found that in only two (neither of which could be followed to complete healing) was the maximum pH lower than 7.4. The highest maximum, that of Case 2, was 7.86, while the lowest (in a case followed only through the initial stages of healing) was 6.96.

We further noted in all cases that the fluctuations in pH followed roughly the clinical condition of the wound; progressive healthy healing being accompanied by a consistent increase in alkalinity, while failure to progress toward healing, or an increase of exudate, was followed by marked fluctuation toward acidity.

The increase in alkalinity beyond the reaction of blood plasma (pH 7.4) might arise from an abnormal reaction of the body tissues to the presence of maggots, or might be furnished by the maggots themselves. Specialized cells in the body are capable of secreting fluids that are relatively strongly acid (gastric juice), or strongly alkaline (pancreatic juice). One would not expect, however, such abnormal secretions from cells and organs not specialized for the purpose. On the other hand, it is known that maggots produce ammonia. Hobson[6] demonstrated its presence in an extract of midguts of blowfly larvae in the absence of ammonia-forming bacteria by means of Nessler's solution. To determine if sufficient ammonia could be formed by maggots to render wounds

alkaline to the degree which we have observed, the ammonia production of sterile blowfly larvae was measured quantitatively.

Air, aspirated through the apparatus illustrated in Fig. 1, passed first through sulphuric acid 1:6, to remove atmospheric ammonia (*A*), then in turn through jar *B* which contained the maggot culture bottle, and over glass wool saturated with 50 per cent sodium hydroxide in *C*. This measure was necessary to prevent excess moisture from condensing in the vertical connections leading out of the incubator, thus trapping ammonia. The air was then rehumidified by bubbling through weak sodium hydroxide solution in *D*, and passed in succession through two absorption tubes, *E* and *F*, each containing 10.0 c.c. of 0.1 N hydrochloric acid.

Larvae of *Lucilia sericata* were grown on sterile homogeneous nutrient slants consisting of casein and agar, together with yeast autolysate to furnish the neces-

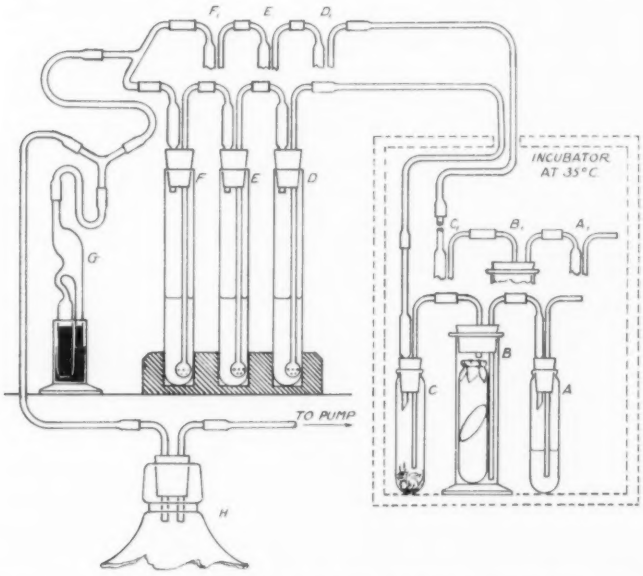

Fig. 1.—Diagram of apparatus used to measure ammonia production of blowfly larvae. (For explanation, see text.)

sary dietary factors required by for growth.* These were contained in 50 ml. centrifuge bottles with constricted necks, the mouths of which were covered with muslin stretched tightly and tied. Lucilia eggs, sterilized by immersion for fifteen minutes in a solution of mercuric chloride, 1:1,000, acidified with hydrochloric acid and washed with sterile water, were seeded on one of these slants, the muslin closure was replaced, and the bottle placed in cylinder *B* in the absorption train. By means of a water pump, air was aspirated through this train continuously for a period of three to six days, during which time the eggs hatched and the maggots developed more or less toward the pre-pupal stage. To equalize fluctuations in vacuum due to variations in water pressure at dif-

*Hobson[9] has shown that blowfly larvae require for growth a food accessory contained in autolyzed yeast. We have found that the liberal addition to synthetic media of a commercial preparation of yeast autolysate, sterilized by filtration through a Seitz filter, gives excellent growth.

ferent times of day and night, it was found necessary to include in the line a mercury valve, *G*, and a five-gallon carboy, *H*, which acted as an air reservoir. A similar train, containing a culture bottle, but without eggs, was placed in parallel as a control (A_1, ... G_1).

At the end of the chosen period both the bottle containing the maggots and the control bottle were removed from the train and acidified with a few cubic centimeters of 0.1 N hydrochloric acid. The maggots were removed by means of forceps, washed, dried on filter paper, counted, and weighed as a group. The washings were added to the culture bottle, which was heated on a water-bath to melt the agar and diluted to 250 c.c. The control bottle was similarly treated. The standard acid in the absorption tubes was titrated, and ammonia nitrogen determined on 25 c.c. aliquots of the solution of the maggot medium and of the control medium, using Sachsse's method[13] of vacuum distillation in the presence of an excess of magnesia.

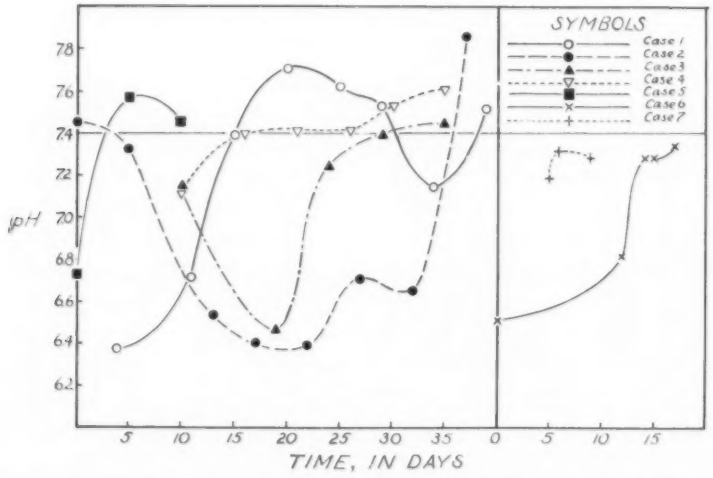

Fig. 2.—Variations in the pH of wound exudates. Cases 1 to 5, chronic osteomyelitis treated with blowfly maggots. Case 6, appendiceal abscess. Case 7, compound fracture of the tibia. Wet dressings of physiologic salt solution only were used in Cases 6 and 7.

Table I shows briefly the result of three experiments of this kind. It will be seen that under sterile conditions, each maggot is capable of furnishing at least 0.1 mg. of ammonia nitrogen per day to its surroundings, and more than this toward the end of its larval existence. The usual wound dressing in osteomyelitis contains at least 100 to 200 maggots, furnishing 10 to 20 mg. of ammonia nitrogen daily. One cubic centimeter of 0.1 N ammonium hydroxide solution, containing 1.4 mg. ammonia nitrogen, is capable of changing the reaction of 2 c.c. of blood serum over approximately one pH unit. Consequently, the amount of ammonia furnished by maggots in the usual dressing would be sufficient to neutralize much of the acid exudate characteristic of inflammation and infection, and, when the latter had to some degree subsided, to account for the excess alkalinity we have observed. It should be pointed out that maggots in the presence of bacteria produce greater quantities of ammonia than when sterile.[8]

The slight excess of alkalinity observed would exert a definite inhibition of bacterial growth in the wound. Since the greater part of the healing takes place while the reaction of the wound exudate is below pH 7.4, this alkalinity cannot be a great factor in early healing, but undoubtedly plays an important part in maintaining bacteriostasis, once the healing has progressed beyond a certain point. To what degree the partial neutralization of the acid inflammatory secretion aids early healing cannot be judged until we know more about conditions influencing the healing process, but it would seem likely to be beneficial to some degree.

TABLE I

AMMONIA PRODUCTION BY BLOWFLY LARVAE

EXPER. NO.	DURATION DAYS	LIVING MAGGOTS RECOVERED	TOTAL WT. OF MAGGOTS GM.	AV. WT. OF ONE MAGGOT GM.	NH_3-N IN ABSORPTION TUBES MG.	NH_3-N FROM MEDIUM MG.	TOTAL NH_3-N PROD. MG.	NH_3-N PER MAGGOT PER DAY MG.
1	4	27	0.5763	0.0231	17.2	Not determined	17.2	0.16
2	6	55	1.8211	0.0331	27.4	20.9	48.7	0.12
3*	3	19	0.9546	0.0328	5.6	30.2	35.8	0.63

*In Experiment 3, partly grown maggots instead of eggs were introduced into the culture bottle. The fact that they were more mature than those used in Experiments 1 and 2 may explain the heightened ammonia production (see Hobson[5]).

The source of the ammonia is not definitely known. Hobson[6] believed that it resulted from the deamination of the amino groups set free as the maggot hydrolyzed its food, but was unable to identify a deaminase in his study of the enzymes of blowfly larvae. Nitrogen distribution studies, still incomplete, have convinced us that Hobson's belief is correct.

THE MECHANISM FOR THE REMOVAL OF NECROTIC TISSUE

All who have watched osteomyelitis wounds healing under treatment with blowfly larvae are impressed with the rapid cleansing of the wound and the resulting pinkish granulation tissue and flat, undistorted scar. Baer, the first proponent of larval therapy, ascribed such results to what he called the "scavenger action" of the maggots;[1] their peculiar food requirements permitting them to attack only dead tissue.

Fabre[3] described the way in which maggots feed upon meat; first liquefying the protein with a proteolytic saliva or excretion, after which they ingest and assimilate the liquid food. Hobson has carried out an investigation of the digestive enzymes of *Lucilia sericata* larvae, and has found a trypsinlike protease in both the digestive tract and excreta,[6] and a collagenase in the excreta.[7] In England, this larva is said to infest sheep, boring into the flesh through the skin. The American variety of the same species, however, was chosen for surgical purposes, because here it is known never to attack living tissue. We have studied the digestive enzymes of *L. sericata* to learn if such a difference in food habits involved a corresponding difference in enzyme mechanism.

Lots of about 500 sterile, half-grown larvae were ground with sand and 50 c.c. of solvent. The ground mixtures were incubated for varying lengths of time at 40° C. and filtered. The proteolytic activities of these extracts were ob-

served toward 10 per cent solutions of casein, gelatin, and peptone, buffered to a pH of 8.0. A modification of the method of Willstätter and Grassman[14] was used to follow the digestion. Sufficient alcohol was added to aliquots of the digest to make 90 per cent alcoholic solutions. They were then titrated with 0.05 N potassium hydroxide in 90 per cent alcohol, from a microburet, using phenolphthalein as an indicator. Under these circumstances, the carboxyl groups freed by proteolysis dissociate, while the corresponding amino groups do not; consequently, the progressive increase in titrable acidity is a measure of proteolysis. Since heating would alter the base-binding capacity of both substrate and enzyme solution, the controls were not heat inactivated. Instead, controls consisting of substrate, and of extract, were incubated in separate tubes, and samples of each titrated along with those of the digestion mixture. From the titration value of the sample of digestion mixture was subtracted the sum of the values for both controls, and the difference gave a figure which was proportional to the degree of proteolysis.

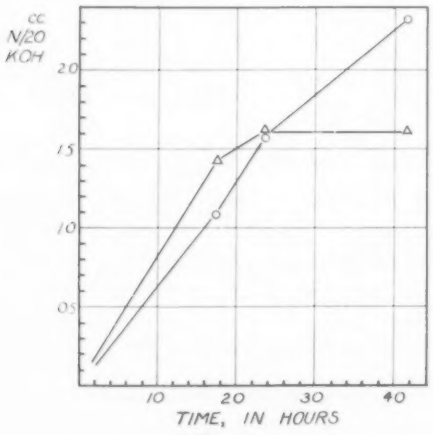

Fig. 3.

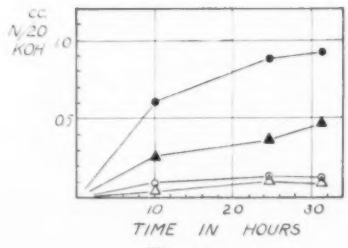

Fig. 4.

Fig. 3.—Proteolytic activity of a 20 per cent glycerol extract of maggots. Ordinates, amounts of KOH necessary to neutralize carboxyl groups liberated. Circles, casein; triangles, peptone.

Fig. 4.—Proteolytic activity of maggot washings compared with that of 1 per cent trypsin. Solid circles, trypsin against gelatin; solid triangles, trypsin against peptone. Hollow circles, washings against gelatine; hollow triangles, washings against peptone.

The best medium for extracting the proteolytic enzymes was found to be 20 per cent glycerol. Eighty per cent glycerol was an inferior solvent, while 0.9 per cent sodium chloride extracted only a trace of active material.

Fig. 3 represents the results of a typical experiment in which crushed maggots were incubated with 20 per cent glycerol for twenty-four hours. By comparison with the digestion rate of Armour's trypsin in Fig. 4, it will be seen that this extract possessed a high proteolytic potency.

In order to obtain a solution of the excretory products of the larvae, we confined lots of maggots in a cylindrical cage of 60 mesh copper gauze and dipped them repeatedly into physiologic salt solution. The cage, containing 200 to 300 maggots, was raised and lowered into 50 c.c. of the salt solution four times per minute for seven hours, by means of a cord passing over pulleys, and

actuated by the motor of a pneumatic windshield wiper. At the end of the seven-hour period, the maggots were all living and vigorous.

The fluid in which the maggots had been immersed more than 1,500 times over a seven-hour period was tested for proteolytic activity against peptone and gelatin, using the same technic as previously described. For comparison, a 1 per cent solution of Armour's trypsin was tested simultaneously. As Fig. 4 shows, the washings, in volume comparable to the extracts described above, showed a tryptic activity barely beyond the limits of error of the method, whereas the trypsin preparation is comparable in potency to the extracts of crushed maggots.

We were not able to identify a collagenase in the solution of excreta, although Hobson appears to have found one in that variety of *L. sericata* common in England. Since such an enzyme would aid the larva to penetrate through the intact skin of an animal, we believe that this difference in enzyme equipment is a reflection of the difference in feeding habits between the English variety of *L. sericata* and that found in America.

We believe our observation that blowfly maggots excrete a weakly proteolytic fluid, while containing in their digestive tracts a relatively strong one, explains why they remove necrotic tissue as effectively as they do. The use in a wound of an enzyme solution sufficiently powerful to hydrolyze the dead tissue completely would be highly irritating. The maggots apparently excrete a fluid just sufficiently proteolytic to liquefy their food, while the bulk of the digestion takes place within their digestive tracts, out of contact with the tissues of the wound. Another important factor in the "scavenger action" is that the proteolytic end-products, which would otherwise be absorbed by the patient's system, or remain in the wound to furnish a substrate for bacteria, are used by the maggots for growth, and thus rendered inert.

DISCUSSION

It is interesting to note that both the alkalinization of the wound and the removal of necrotic tissue by maggots depend on the presence of living larvae. Similarly, Robinson and Norwood[11] have shown that maggots are capable of ingesting and destroying by digestion large numbers of bacteria. These facts demonstrate the impossibility of substituting for the living organism extracts or pastes prepared from blowfly larvae, as has been suggested in the past.[5, 10]

Indeed, it is reasonable to suppose that much of the unique virtue of maggot therapy depends upon minuteness and thoroughness of action, and the uniformity with which the concentration of any active agent which the larvae may excrete may be maintained in the wound. Compared with these conditions, which are best realized in the presence of large numbers of minute active agents, surgical instruments and bactericidal dressings are relatively crude tools.

SUMMARY AND CONCLUSIONS

1. Chronic osteomyelitis wounds, healing in the presence of blowfly larvae, develop reactions more alkaline than pH 7.4, in contrast to wounds dressed only with physiologic salt solution.

2. It has been shown that sterile *Lucilia sericata* larvae produce sufficient ammonia to account for this excess alkalinity.

3. The excess alkalinity is probably a factor in bacteriostasis and wound healing.

4. Blowfly larvae are shown to excrete a relatively weak proteolytic enzyme, while they contain in their digestive tract a more powerful one.

5. The relative strength and location of these enzymes permit the removal of necrotic tissue from a wound with a minimum of irritation.

6. The assimilation by the larvae of the protein split products of necrotic tissue removes the latter from the wound where they would otherwise putrefy or be absorbed, to the detriment of the patient.

7. Maggot therapy depends for its beneficial action on the presence of living larvae, which cannot be successfully replaced by pastes or extracts of maggots.

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Note: Since the above was written, Dr. William Robinson (J. Bone & Joint Surg. **17**: 267, 1935) has pointed out that allantoin, present in the excreta of maggots, stimulates cell proliferation, and recommends its use to aid the healing of indolent wounds and ulcers. If such treatment proves successful, one function of maggot therapy, the stimulation of rapid healing, can be carried out in the absence of living larvae by means of a larval catabolite. We affirm our last conclusion, however, with respect to the two functions of maggot therapy which we have particularly studied: the removal of necrotic tissue, and whatever degree of bacteriostasis may be effected.

THE PRIMARY CARCINOMA OF THE LUNG*

A Review of 100 Autopsies

R. H. Jaffé, M.D., Chicago, Ill.

IN RECENT years numerous reports have appeared from different parts of the world which deal with the increase in frequency of the primary carcinoma of the lung. Formerly considered a rare disease, seldom correctly diagnosed during life, modern statistics give the incidence of primary carcinoma of the lung with from 8 to 20 per cent of all carcinomas. Much controversy has arisen as to the cause of this high incidence of pulmonary carcinoma compared with the incidence of two or three decades ago. Many authors emphasize the great importance of the persistent irritation of the lungs by dust and gases which are the result of modern life and which are known to contain carcinogenic substances. Thus, by tar, pulmonary carcinoma has been produced experimentally in mice. Carcinoma of the lung, however, is also very common in countries in which the tarring of roads is but little used and automobiles are few in number (e.g., Russia). Other investigators stress, therefore, the significance of pulmonary infections, in particular the last influenza pandemic and focal, chronic pneumonias which seem to be more common than in former years. On the other hand, there is a group of authors who believe that the increase in frequency of pulmonary carcinoma is merely due to the fact that we have learned to recognize it better, and that with the increasing span of life more people reach the age in which carcinoma of the lung is most common.

Whether its increase in frequency is real or apparent, carcinoma of the lung, undoubtedly, is a common disease and a thorough familiarity with it is of importance. The great progress in thoracic surgery and in radiotherapy makes one hopeful that, if diagnosed early, pulmonary carcinoma is not an absolutely fatal disease, and an increasing number of reports are being published which suggest a complete cure or, at least, considerable prolongation of life by surgery or radiation. The early diagnosis of a disease is intimately linked to the knowledge of its anatomical manifestations. The modern literature contains many studies on the pathology and histogenesis of pulmonary carcinoma and, in addition to the chapters in the textbooks on malignant neoplasms (Ewing, Stout), I refer to the monographs and articles respectively of W. Fischer, Fried, Hruby and Sweany, Huguenin and Weller. Personal experience with a large number of cases has the advantage of uniform analysis and justifies the publication of additional material. It is with this point of view in mind that I present the following review of one hundred cases of primary carcinoma of the lung which have come under my observation during the past six years and which have been proved at autopsy and by microscopic examination.

*From the Department of Pathology of the Cook County Hospital.
Received for publication, March 15, 1934.

Frequency.—My material consists of 6,800 autopsies, 871 of which have been on cases with carcinoma. With *11.47 per cent* carcinoma of the lung is third in frequency among the carcinomas, carcinoma of the stomach being first with 184 cases, and carcinoma of the intestine being second with 118 cases. In 1921, I published, from a different part of the world (Vienna, Austria), statistics which were based upon 4,500 autopsies performed between 1915 and 1918. In these statistics the incidence of pulmonary carcinoma among the carcinomas was 10.73 per cent. It is, of course, not permissible to compare figures which have been collected under entirely different conditions, but the similarity between the two figures is so striking that I have thought them worth while quoting. I am inclined, therefore, to believe with Fried and others that the increase in frequency of primary carcinoma of the lung is more apparent than real.

Etiology.—My material does not offer any definite suggestion as to the probable etiology of pulmonary carcinoma. The patients came from the most different walks of life. Fine and Jaso and others assume that silicosis may predispose to pulmonary carcinoma. Among my hundred cases there was only one with the anatomic evidences of silicosis and in this case the silicon content of the lung amounted to 4.79 mg. per one gram dried lung tissue. Allen believes that in the coal miners of Pennsylvania carcinoma of the lung is not more common than in the other population. In a critical review Saupe comes to the conclusion that the high incidence of pulmonary carcinoma in certain mining districts is due rather to radioactive substances in the air of these mines than to anorganic dust. The tendency of syphilis to cause chronic irritation of the mucous membranes with epithelial metaplasia and carcinomatous transformation of the metaplastic epithelium has often been used as an argument that syphilis may play an important rôle in the pathogenesis of carcinoma of the lung. Twenty per cent of my cases of carcinoma of the lung showed anatomic or serologic evidences of syphilis which is distinctly higher than in the rest of my material of individuals over twenty years of age (approximately 11 per cent). L. Popper states that 12 per cent of his cases of bronchiogenic carcinoma have shown evidences of syphilis while in 337 cases of other carcinomas this combination has been found in only 3.9 per cent. In seven instances I have found, in addition to the carcinoma, a progressing tuberculosis of the lungs. The anatomic findings suggested that the carcinoma had stimulated to active progression a silent preexistent tuberculosis. There was no case of carcinoma formation from the metaplastic epithelium of an old tuberculous cavity.

None of my cases showed definite relations to a preceding trauma. There was one case of carcinoma of the lung in a colored man, forty-three years of age, who, several years prior to his death, had received a bullet wound to the chest. At autopsy the bullet was found, well encapsulated, in the right lower pulmonary lobe, while the left lung showed a squamous cell carcinoma that had originated from the main bronchus. I think that these findings exclude the possibility that the old injury may have been related to the tumor. In general, it may be said that in the majority of the cases, at the time of death, the tumor has advanced so far as to obscure any preceding or predisposing local changes.

Age, Sex and Race.—All statistics agree that carcinoma of the lung is most common in the fifth decade of life and that it is much more frequent among males than among females. According to the age my hundred cases are distributed as follows:

Below 30 years (youngest case 24 years)	2
31-40 years	6
41-50 years	30
51-60 years	41
61-70 years	16
Over 70 years	5

Ninety-two per cent were in males. Racial differences were not observed. The total incidence of pulmonary carcinoma was higher in the white race than in the colored race, namely, 1.7 per cent of all autopsies in the white race and 1.0 per cent of all autopsies in the colored race. This difference is of no significance because the average span of life of my material is about ten years lower in the colored race than in the white race. In the colored race the difference between males and females was less striking than in the white race, 35 per cent of the tumors being found in negresses.

Location.—In most of the statistics the right lung has been found to be more frequently involved than the left lung. In some statistics the opposite is the case (e.g., Kraft, Edwards). In my material the relation between carcinoma of the right and left lung is 55:45. Fifty-five tumors involved the upper lobes and 33 tumors involved the lower lobes. There was only one carcinoma of the right middle lobe. In eleven cases the tumor had originated from the main bronchus proximal to its first division.

Macroscopic Appearance.—The gross appearance of the tumor depends chiefly upon the extent of the local progression. If local complications or the metastases cause the patient's death at a relatively early stage of the disease, the primary neoplasm in the lung may be so small that it is easily overlooked at autopsy. In these cases, one finds, in a larger or smaller bronchus, a circumscribed, indurated area of the mucosa with or without slight infiltration of the surrounding lung tissue. In some cases a small and friable polyp may protrude into the lumen of a bronchus. If such a polyp has become sequestrated and expectorated, there may be a small and shallow ulcer, the true nature of which can be recognized only on histologic examination. More frequently, the tumor has grown to a more or less circumscribed nodule or to a large node surrounding a bronchus and compressing its lumen. There are cases of pulmonary carcinoma which present themselves as diffuse consolidations of part of a lobe or of an entire lobe, resembling a lobular or lobar pneumonia. In some instances regressive changes have transformed the tumor into a huge cavity with a thin lining of intact tumor tissue. Most carcinomas of the lung are soft, medullary in consistency and pale gray or grayish white in color.

For practical purposes Rabin and Neuhof distinguish two main groups of cancer of the lung. One group is called the noncircumscribed type. This type to which three-fourths of the tumors belong originates from the main bronchus

or from a branch bronchus and reveals the usual invasive characteristics of cancer. The second group is called the circumscribed type and presents itself as a spherical, well-demarcated growth macroscopically unrelated to a bronchus. Cancers of the circumscribed type occupy the parenchymal and peripheral zones (parenchymal and peripheral tumors). Geschickter and Denison also refer to two groups and speak of a hilar or epidermoid carcinoma that is most common and occurs at the center of the lung, and of a lobular carcinoma that arises in the periphery of the lung. I have divided my material of pulmonary carcinoma into four types, namely, the central or hilus, the intermediary, the peripheral and the diffuse type. The central type, which starts from the main bronchus or its first divisions and quickly spreads to the hilus and mediastinal lymph nodes

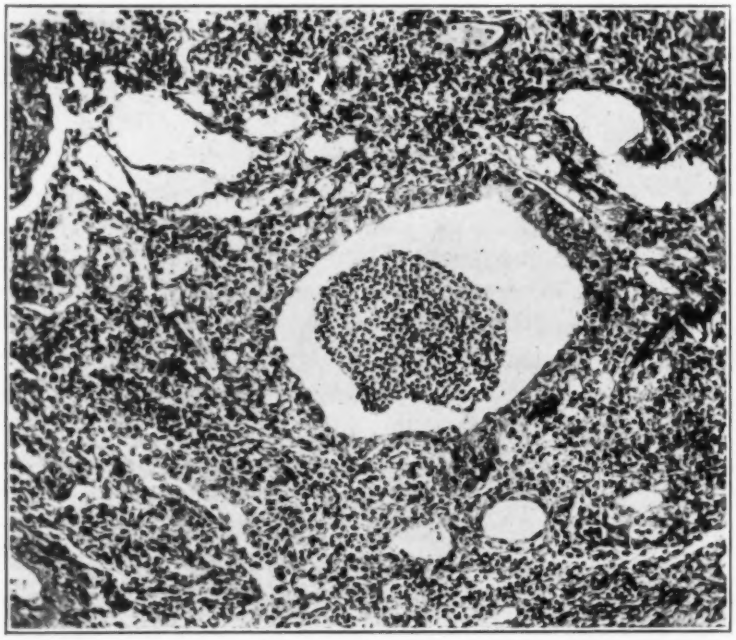

Fig. 1.—Peripheral, chronic interstitial pneumonia. In the center of the field is a small bronchus which is lined by metaplastic epithelium. There is some irregularity in the arrangement and shape of these cells. The lumen of the bronchus contains clumps of pus cells. Mallory's phosphotungstate hematoxylin stain, magnification ×150.

fusing with them to huge solid masses, is the most common type. It was found in 76 per cent of my cases. With 13 per cent the peripheral type is next in frequency. It starts in the periphery of the lung underneath the visceral pleura. In a case which is not included in my statistical figures a peripheral carcinoma was discovered at autopsy as an incidental finding. The patient was a white man, fifty-nine years of age, who for many years, in spite of all treatments, had a persistently strongly positive Wassermann reaction and who died from decompensation of a hypertrophic heart. The lower border of the right lower lobe felt firm and nodular. On microscopic examination there was a marginal, chronic, interstitial pneumonia with extensive metaplasia of the epithelium of the smallest bronchi into squamous epithelium (Fig. 1). In several places the metaplastic epi-

thelial cells appeared very irregular with hyperchromatic, anaplastic nuclei and atypical mitotic figures. In one area there was a distinct invasion of the connective tissue surrounding the bronchus and lymph vessels and small veins were found filled with the atypical cells (Fig. 2). In another case in which the patient died from a hemorrhage into a cerebral metastasis a small primary tumor was found in the apex of the left upper pulmonary lobe.* With its necrotic center the primary tumor resembled somewhat an apical tubercle except for the medullary, grayish white tissue about the necrosis. The histologic examination disclosed a very immature round-celled carcinoma. The tumor cells were arranged in small alveoli. According to my experience the peripheral carcinoma offers the greatest diagnostic difficulties. It may spread diffusely over the pleural surface of the

Fig. 2.—This area shows the invasion of the stroma by the atypical cells, some of which are found in the lumen of lymph vessels. The cells also extend into and line the two alveoli seen at the left and right side of the field. Staining and magnification same as Fig. 1.

whole lobe or over the entire lung encasing it into a curasse of tumor tissue. These cases are often mistaken for mesotheliomas of the pleura, a tumor which is exceedingly rare. In the central type of pulmonary carcinoma this diffuse pleural spreading is less common. In six cases the tumor was located in the center of a lobe originating from a bronchus of third, fourth, or fifth order (intermediary type). Finally, there were five cases with diffuse involvement of a lobe.

Associated Changes in the Lung.—In many instances the carcinoma leads to secondary changes which may be restricted to the lobe that is the site of the

*According to the observations of Steiner and Francis the "superior pulmonary sulcus tumor" described by Pancoast corresponds most likely to an apical carcinoma of the lung.

tumor or which may involve the entire lung. These secondary changes are, sometimes, so marked that they obscure the tumor. In thirty-six cases the carcinoma of the lung was associated with diffuse or saccular bronchiectases and the relation between the tumor and the bronchiectases indicated that the widening of the bronchial lumen was secondary to the tumor stenosis. In only one case did the carcinoma originate from the epithelial lining of an old bronchiectatic cavity. There were 17 cases of chronic pneumonia distal to the tumor, 8 cases of lung abscess and 4 cases of lung gangrene. The abscesses and gangrenous areas involved portions of the lung peripherally to the tumor.

Changes of the Pleural Cavity.—Spreading along the peribronchial lymph vessels the carcinoma often reaches the pleural cover causing reactive changes of the pleura. Recent exudative processes were encountered in 43 of my cases. The character of the exudate was as follows: serous, 14 cases; hemorrhagic, 13 cases; suppurative, 12 cases; fibrinous, 4 cases. There were 26 cases of partial and 23 cases of complete obliteration of the pleural cavity. In 8 cases the pleura did not show any changes.

Compression and Invasion of Adjacent Structures.—It is particularly the hilus type of pulmonary carcinoma with its tendency to spread toward the mediastinum which leads to the involvement of the neighboring organs. In seven cases the tumor had invaded and obliterated the superior vena cava, causing the formation of extensive collaterals in the anterior wall of the chest. In two cases the inferior vena cava was found obliterated by tumor tissue. There was one case each of invasion of the trachea and esophagus and three cases of direct extension of the tumor through the pericardial sac into the myocardium of the right auricle. Compression of the trachea was encountered twice and compression of the esophagus four times. In all these cases the metastases to the tracheobronchial and mediastinal lymph nodes had fused with the pulmonary tumor into a solid mass. There were four instances of erosion of a larger pulmonary vein with severe hemorrhage. In one case the pulmonary tumor had grown through the foramina intervertebralia into the spinal canal compressing the midportion of the dorsal cord. One peripheral carcinoma had extended through the intercostal spaces into the pectoral muscle impressing, at first, as a sarcoma of the major pectoral muscle.

Metastases.—The carcinoma of the lung is characterized by a most extensive hematogenous and lymphatic spreading. It has already been mentioned that an insignificant primary tumor may be found in cases of generalized metastases. Because the pulmonary carcinoma sometimes presents itself under the picture of a primary tumor of the brain, French authors refer to a cerebral form of carcinoma of the lung. With the same justification one could speak of a gastric, intestinal, osseous or lymph node form of the pulmonary carcinoma. Compression of the duodenum by metastases to the peripancreatic lymph nodes, or compression of the cardia of the stomach by metastases to the perigastric lymph nodes, or compression of the common duct by metastases to the peribiliary lymph nodes often directs the clinician's attention to this region of the body. In only

two of my hundred cases no metastases were found at autopsy. The other cases showed the following distribution of the metastases:

Thoracic lymph nodes	89 times	Pancreas	6 times
Other lung	43 times	Spleen	5 times
Adrenals	42 times	Peritoneum	4 times
Abdominal lymph nodes	37 times	Dura mater of spinal cord	3 times
Liver	36 times	Skin, thyroid, pericardium, muscles	2 times
Kidneys	28 times	Stomach, seminal vesicles, tongue, ovary, testicle, breast	1 time
Bones	22 times		
Brain	19 times		
Intestine	8 times		
Heart	7 times		

The figures for the metastases to the brain and bones are apparently too low, since in twenty-three cases no permission was obtained for removing the skull, and a systematic examination of the bones could not be made. Schmorl points out that in 33.3 per cent of the cases with pulmonary carcinoma metastases can be found in the spine.

Histopathology.—As source of the primary epithelial neoplasm of the lung the surface epithelium of the bronchi, the epithelium of the mucous glands of the bronchi and the alveolar epithelium have been considered. As far as the alveolar epithelium is concerned it appears doubtful whether the lung of adult human beings possesses an epithelial lining. The majority of the modern investigators trace the pulmonary carcinoma chiefly to the bronchi (Kraft, Sweany, Fried, Fischer, Tuttle and Womack and others). They are, particularly, the short, dark nucleated epithelial cells which are wedged in between the basal portion of the ciliated epithelial cells and which have been designated as basal cells from which the carcinoma of the lung seems to originate. These basal epithelial cells supply the material for epithelial regeneration and are endowed with the prosoplastic potentialities to differentiate into ciliated or nonciliated columnar cells, mucus-producing epithelial cells and squamous epithelial cells. The carcinoma of the lung shows great variations in the microscopic structure and different areas from the same tumor may reveal widely different pictures.

In thirty-one of my cases the tumor was composed of undifferentiated, round cells with scanty cytoplasm and round nuclei rich in chromatin. The cells varied from six to twelve microns in diameter and formed solid areas which were surrounded by a scanty stroma with thin-walled capillaries. In the center of the solid areas necrosis was common. This type of tumor has been called round-celled or solid carcinoma, and its resemblance to sarcoma has often been stressed. In 28 cases the tumor cells were distinctly larger and more pleomorphic and possessed an ample cytoplasm. The shape was polygonal, elongated, or oval, and the nuclei contained a compact chromatin net and prominent nucleoli. In some of the tumors huge giant cells were encountered. These cells arranged themselves in a pavement-like fashion resembling closely the undifferentiated squamous cell carcinoma of the mucous membranes. In Fischer's statistics too, squamous cell carcinoma was second in frequency. Some authors refer to this type of carcinoma of the lung as pleomorphic celled (Kraft and others). Among the squamous cell carcinomas there were three with hornification and numerous

horn pearls. Since the basal cells are potentially able to differentiate into squamous epithelium, the high incidence of squamous cell carcinomas of the lung is not surprising. Some cases suggest, however, that the squamous cell carcinoma may develop from a previously metaplastic epithelium. I have repeatedly seen in cases of squamous cell carcinoma of the lung areas of metaplasia of the bronchial epithelium far away from the tumor. There were twenty-four instances of adenocarcinoma in three of which the formation of papillae was an outstanding feature. Whether the tumor glands are derived from the surface epithelium or from the epithelium of the mucus gland I cannot decide. In three cases of mucus-producing carcinoma an origin from the goblet cells of the surface epithelium or from the mucus glands may be considered. The type of pulmonary carcinoma which is characterized by the spindle shape of the cells and which, under the term "oat celled" carcinoma, has been discussed especially by British authors (Barnard and others), has been encountered eight times. The types of tumors discussed so far did not differ in their gross appearance nor were there any differences in the duration of life or the extent of metastases.* In four cases the tumor was firmer and on microscopic examination small alveoli of cuboidal or cylindric cells were found which were embedded by an ample stroma. These cases were called carcinoma simplex. In two cases in which the primary tumor was very small and appeared as a firm, circumscribed thickening of the bronchial mucosa, the histologic picture was that of a scirrhus. Both carcinoma simplex and scirrhus had produced extensive metastases.

Duration.—In a disease in which the onset is as insidious as in pulmonary carcinoma, it is impossible to determine the duration of life after the tumor has started to develop. The following table is based upon the appearance of the first symptoms which are probably due to the pulmonary lesion. Seven cases are excluded because the patients died soon after admission without adequate history.

Less than 1 month	3 cases
1 to 3 months	30 cases
4 to 6 months	24 cases
7 to 12 months	26 cases
Over 1 year	7 cases
Over 2 years	3 cases

In fifty-seven cases the duration of the illness was less than six months while in only ten cases did the patient live a year or longer. I did not find any definite relation between the size of the primary tumor or the extent of the metastases and the duration of the illness. From the statistics of Tuttle and Womack it appears that the average duration of life is shorter in the cases with the peripheral type of pulmonary carcinoma than in the cases in which the tumor originates in major bronchi. In my material the average duration of life was eight months in the central type and five months in the peripheral type.

First Symptoms.—It is not the purpose of this paper to enter into a discussion of the clinical aspects of pulmonary carcinoma. A summary of the first symptoms, however, may be given since I have found them a frequent source

*I fully agree with Tuttle and Womack and others that the grading of bronchiogenic carcinoma on histologic grounds is of little practical value.

of confusion. On admission 54 patients complained chiefly of weakness and loss of weight. The loss of weight varied between 10 and 60 pounds. Pain in the chest was encountered 34 times and was often described as sharp and shooting. In 54 cases a persistent cough was among the first symptoms, and in 22 cases the cough was said to be productive of a blood-streaked material. Twenty-eight patients referred to shortness of breath and 5 patients complained of difficulties in swallowing. There were 3 cases in which hoarseness was the first symptom. Twenty-four patients showed no subjective symptoms pointing toward the chest. Fourteen patients complained of abdominal or epigastric pain, 10 patients of loss of appetite, nausea, and vomiting. Five patients referred to constipation. Hess and Faltischek have discussed persistent constipation in cases of bronchial carcinoma. This constipation affects mainly the proximal portion of the colon and has been attributed to the involvement of the right vagus by a hilus tumor of the right lung. In 13 cases the symptoms pointed to the central nervous system.

Diagnosis.—My study is based upon the material of a large charity hospital and many patients enter the hospital in an advanced stage of the disease. Their average stay in the hospital is short and language difficulties are often encountered. Some of the patients with pulmonary carcinoma were too ill for a thorough clinical, bronchoscopic or roentgenologic examination. The longer the stay in the hospital the higher also was the percentage of correct diagnoses. Taking all cases together 59 were correctly diagnosed as pulmonary carcinoma. In the first three years 47.5 per cent were missed while in the last three years, after many cases had been shown in the pathologic-anatomic demonstrations, the percentage of missed diagnoses dropped below 30 per cent. The highest incidence of incorrect diagnosis was found in the peripheral type of pulmonary carcinoma, namely 46 per cent. Of the incorrect diagnoses carcinoma of the stomach is of particular interest. This diagnosis was made in nine cases of carcinoma of the lung. In these cases extensive metastases were found in the perigastric or peripancreatic lymph nodes, compressing the cardiac or pyloric portion of the stomach or the duodenum. In six cases the metastases had perforated into the lumen of the stomach or duodenum, causing confusing x-ray findings and the presence of occult blood in the feces. The other incorrect diagnoses were:

Pulmonary tuberculosis	9 cases
(Five of these cases showed tubercle bacilli in the sputum and were found, at autopsy, to be combined with active tuberculosis)	
Lung abscess	3 cases
Decompensated heart	1 case
Brain tumor or central nervous system syphilis	6 cases
Sarcoma of bone (metastasis to sternum)	1 case
Carcinoma of breast (metastasis to breast)	1 case
Hodgkin's disease	1 case
Aleucemic lymphadenosis	1 case
In these two cases there were generalized metastases to the peripheral lymph nodes and patients refused the taking of biopsies	
Carcinoma of the prostate	2 cases

In seven cases no diagnosis was made because the patient died soon after admission.

In this connection it may be mentioned that extrapulmonary carcinomas may present the clinical and also the anatomic picture of a primary carcinoma of the lung. Thus, I observed two cases of carcinoma of the prostate which, at autopsy, were thought to be carcinomas of the lung. In these two cases there was a single large metastasis to the lung which had perforated into an adjacent bronchus and branch of the pulmonary vein with extensive secondary, hematogenous dissemination to many organs, especially to the adrenals, kidneys, and brain. The prostate was slightly enlarged and soft but on microscopic examination a typical carcinoma of the prostate was discovered.

Value of Biopsy in Pulmonary Carcinoma.—Tumors of the large bronchi which can be seen on bronchoscopic examination lend themselves to biopsies. According to C. L. Jackson bronchoscopic biopsy will be positive in about 75 per cent of the cases of bronchiogenic carcinoma. In taking bronchoscopic biopsies one has to be careful to select the proper area, avoiding necrotic tissue and noncharacteristic reactive changes peripherally to the tumor. Without positive bronchoscopic findings there are other possibilities to confirm the diagnosis by a biopsy. I am referring especially to the high incidence of metastases to peripheral lymph nodes. In twenty-eight of my cases enlarged lymph nodes could be palpated during life. The following lymph nodes were found to be involved:

Cervical and supraclavicular	26 times
Submaxillary	3 times
Axillary	4 times
Inguinal	2 times

Sputum and pleural effusion yield valuable material for histologic examination. They can be precipitated and fixed with alcohol and embedded with paraffin. In two of my cases with skin metastases biopsies from the skin directed the clinician's attention to the lung. Finally biopsies from bone metastases and from the lung and pleura with the aid of an exploratory thoracotomy may be mentioned.

Extrapulmonary Complications.—Eight cases of pulmonary carcinoma showed, at autopsy, an exudative pericarditis. In three cases a terminal endocarditis was found. There were two cases of purpura hemorrhagica and two cases of severe jaundice due to the compression of the common duct by metastases to the peribiliary lymph nodes. There was one case of amyloidosis and only one case of hypertrophic osteo-arthropathy (Marie-Bamberger). Four cases showed a very marked clubbing of the fingers. In ten cases the spleen was palpable during life.

Other Primary Tumors of the Lung.—Apart from carcinoma primary tumors of the lung were very rare in my material. There was one case of benign, glandular polyp and two cases of chondroma, one of which had developed in the wall of a bronchiectatic cavity. There was one case of mesothelioma of the pleura but no case of true sarcoma of the lung. In six of thirty cases of Hodgkin's disease the lungs were found to be extensively involved. It may be

recalled that Hodgkin's disease of the lung may so closely resemble pulmonary carcinoma that differentiation is possible only by histologic examination.

SUMMARY

The autopsy findings of one hundred cases of primary carcinoma of the lung are reviewed. Except for a high incidence of syphilis no suggestive etiologic factor was recorded. The great predominance of the male sex was confirmed. Racial differences were not observed. Whether carcinoma of the lung is really increasing in frequency appears doubtful.

Macroscopically a central, intermediary, peripheral and diffuse type of pulmonary carcinoma have been distinguished. The peripheral type seems to offer the greatest diagnostic difficulty.

The majority of the pulmonary carcinomas are composed of undifferentiated round cells which are apparently derived from the basal cells of the bronchi. The high incidence of squamous cell carcinoma suggests relations to epithelial metaplasia. Other types of pulmonary carcinoma are: the adenocarcinoma, the mucus carcinoma, the carcinoma simplex, and the scirrhus.

The differential diagnostic difficulties which are due to the early appearance of metastases to distant organs are discussed. The value of biopsies is emphasized.

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III. LOCAL CEREBRAL ANAPHYLAXIS IN THE DOG*

LEO M. DAVIDOFF, M.D., NICHOLAS KOPELOFF, PH.D., AND LENORE M. KOPELOFF, M.A., NEW YORK, N. Y.

IN ORDER to augment the material presented in previous communications[1] and particularly to increase the number of control animals, a further study of twenty-three dogs was undertaken. These dogs varied in weight between 4 and 14 kg., the average being 8 kg. Two methods of sensitization were employed. The first consisted in placing the antigen in a small collodion sac in contact with the cerebrum, which was either purposely injured by scarification or left comparatively intact. In control animals the sacs were filled with sterile physiologic saline. In the second method, on five successive days 0.2 c.c. of antigen was injected directly into the brain through a previously prepared trephine hole after allowing the wound to heal. Control animals received similar injections of saline.

The two antigens used were normal horse serum,† which was always employed on the left side of the brain, and fresh egg white, which was always used on the right side of the brain. The sacs were filled with 0.05 c.c. of equal parts of horse serum and saline, or 0.10 c.c. of 17 per cent egg white (diluted with saline). The test for sensitivity consisted of an injection into the leg vein of 4 c.c. of undiluted normal horse serum or egg white.

In Tables I to III the values assigned to the general reaction represent degrees in terms of the number of responses noted, i.e., diarrhea, vomiting, weakness, salivation, and death. The degree of intensity of the focal reaction is indicated as very slight (±), slight (+), moderate (++), marked (+++), and very marked (++++). It goes without saying that any focal reaction observed occurred on the side of the animal opposite the side of the brain which was in immediate contact with the antigen.

HORSE SERUM

Considering first the dogs which received horse serum alone, we found that after the first intravenous injection (No. 52, sac without cerebral injury), there occurred a very marked contralateral paralysis accompanied by an extremely severe general reaction terminating in death one-half hour after the injection of horse serum. Another dog (No. 62, sac with injury) did not react immediately to its first intravenous injection of horse serum, but three hours later salivated, rotated its head to the right, showed convulsive twitching of the right forepaw, and backward stamping of the right hind leg. Control dogs (saline sac in contact with the brain) did not react in any way to the first intravenous dose

*From the Department of Bacteriology, New York State Psychiatric Institute and Hosp'tal.

†This was very generously placed at our disposal by the New York City Board of Health Research Laboratories.

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of horse serum. Upon repeated intravenous injections, occasional reactions, usually general in character, occurred. One dog, however (No. 51, sac without

TABLE I

RESULTS OF INTRAVENOUS INJECTION OF HORSE SERUM

		HORSE SERUM TREATED								CONTROLS					
DOG. NO.		52		62		53		70		51		63		73	
PREPARATION		HS SAC		HS SAC INJURY		HS INJECTED		HS INJECTED		SALINE SAC		SALINE SAC INJURY		SALINE INJECTED	
NO. WEEKS AFTER PREPARATION	ANTIGEN I. V.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.
3	HS	++++	++++							0	0				
7	HS			*+	++++							0	0		
8	HS					0	0	0	0					0	0
11	HS			++	0							+++++			
12	HS									0	0				
17	HS					0	0	++	+++					0	0
21	HS			+	0					++	+				
26	EW					0	0	0	0						
27	HS					++	0	+	0					0	0
29	EW									0	0				
30	HS			0	0					++	0				
37						0	0	+++	0						
39				0	0					+	0				

*Delayed reaction, four hours after I.V. injection. EW, Egg white. Loc., Local reaction. HS, Horse serum. Gen., General reaction.

TABLE II

RESULTS OF INTRAVENOUS INJECTION OF EGG WHITE

		EGG TREATED				CONTROLS					
DOG NO.		57		68		58		64		72	
PREPARATION		EW SAC		EW INJECTED		SALINE SAC		SALINE SAC INJURY		SALINE INJECTED	
NO. WEEKS AFTER PREPARATION	ANTIGEN I. V.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.
7	EW	0	0			0	0	0	0		
8	EW			++	0						
9	EW									0	0
11	EW							0	0		
12	EW	0	0			++	0				
18	EW			+++	0					0	0
21		++	0			+++	++	++	0		
27				+++	0					0	0
30		+++	0			+	0	++	0		
35				+	0						
39		+	0			+	0	0	0		

injury), exhibited a slight focal reaction after the third intravenous injection of the antigen. Although in the initial operative procedure the cerebrum was not purposely injured, undoubtedly a certain degree of trauma occurred in the

parts adjacent to the sac. It is not unlikely that in just this area a concentration of antigen following intravenous injection occurred which was tantamount to sensitization. A subsequent intravenous injection of antigen could therefore elicit a focal reaction, as indeed it did.

As a further control procedure, 3 dogs (No. 53 and 70, horse serum injected, and No. 58, saline sac without injury) received intravenous injections of *egg white* following at least two previous intravenous injections of horse serum. None of these dogs reacted to this first contact with a new antigen. Subsequent intravenous injections of horse serum caused general reactions, thereby demonstrating the specificity of the responses observed.

It is also of interest to note that after the second intravenous injection of horse serum No. 70 exhibited a marked reddening of the scalp, accompanied by itching, indicating a sensitization of the skin in this region. This phenomenon was likewise observed in another dog sensitized to egg white (No. 40) included in a previous series of animals.[2]

EGG WHITE

Among the dogs receiving egg white in the brain, none exhibited a local reaction following the first intravenous injection of the same antigen. Neither did any of the control, or saline-treated animals. However, No. 68, which had received egg white directly into the brain by injection, showed a moderately severe generalized anaphylactic reaction.

Upon repeated intravenous injections of egg white, all but one of the control dogs responded with general reactions. Our interpretation of this phenomenon is the same as that in the case of the animals reacting to repeated intravenous injections of horse serum. Namely, the first intravenous injection of antigen may act as a sensitizing dose by selective adsorption in traumatized brain tissue. Subsequent intravenous injections therefore may cause a local reaction. The protocol of No. 58 (saline sac without injury) is illustrative. After the first intravenous injection of egg white there was no reaction; after the second intravenous injection there was a moderate general reaction (++); after the third intravenous injection there was a severe general reaction (+++) and a moderate *local* reaction (++).

The failure to obtain any reaction in the dogs with egg white sacs following the first intravenous injection of antigen indicates that the antigen may not have come in contact with brain tissue, i.e., that the sac was impermeable to the protein employed. It was very difficult to prepare collodion sacs of such small size (0.3 c.c. capacity) as seemed desirable to use. Furthermore, it should be noted that they were not all tested prior to use as they should have been. The failure to react to intravenous injections by dogs which had received egg white intracerebrally can be accounted for by assuming that there was insufficient contact of antigen with brain tissue to ensure sensitization, i.e., that the antigen was eliminated too rapidly from the local site.

HORSE SERUM AND EGG WHITE

Turning now to a consideration of the dogs which received both horse serum and egg white on opposite sides of the brain, we find that one dog (No. 67,

TABLE III

RESULTS OF INTRAVENOUS INJECTIONS OF HORSE SERUM AND EGG WHITE

		HS AND EW TREATED										CONTROLS			
DOG NO.		54		66		55		65		67		61		71	
PREPARATION		HS SAC EW SAC		HS SAC EW SAC		HS SAC EW SAC INJURY		HS EW INJECTED		HS EW INJECTED		SALINE SAC INJURY		SALINE INJECTED	
NO. WEEKS AFTER PREPARATION	ANTIGEN I. V.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.
3	HS	++++	±			+++	0								
7	HS											0	0		
	EW	++	0			0	0					0	0		
8	HS							+	0	+++	0			0	0
9	EW							0	0	+	+++			0	0
10	HS			0	0										
11	HS			±	0							±	0		
12	HS	+	+												
	EW			0	0							0	0		
13	EW	+	0												
16	HS					0	0								
17	HS							+	±	+	++				
	EW					++	0								
18	EW							++	±	0	0				
	HS													+	0
19	EW													0	0
20	HS			0	0							0	0		
21	EW			0	0							±	0		
22	HS	++	++												
23	EW	+	0												
26	HS					+	0	+	+	++	0				
27	EW					++	0	+++	+	0	0				
28	HS			0	0									++	0
	EW													+	0
29	HS											+	0		
	EW			0	0										
30	EW											0	0		
31	HS	+++	0												
32	EW	0	0												
35	HS					+++	0								
36	HS							±	0	0	0				
	EW					+	0	+	0	0	0				
39	HS			0	0							0	0		
	EW			0	0							0	0		
41	HS	0	0												
	EW	++	0												
45	HS					0	0								
	EW					0	0								

intracerebral injections) responded with a marked contralateral focal and slight general reaction to its first intravenous injection of egg white. Another dog (No. 54, sacs without injury), after its first intravenous injection of horse serum, exhibited a focal reaction of very slight degree accompanied by a severe general reaction. Control animals, saline-treated, gave no reaction to their first intravenous injection of either antigen. Subsequent intravenous injections of both antigens produced focal and general reactions in all but one dog in this group. An interpretation of this phenomenon has already been suggested.

DISCUSSION

The sensitization of brain tissue to horse serum or egg white is beset with serious technical difficulties. Positive evidence of *local* as well as general cerebral anaphylaxis has been presented in detail. Manifest reactions following the *first* intravenous injection of the appropriate antigen in the brain are conclusive. Reactions following repeated intravenous injections of the appropriate antigen in dogs subjected to the brain operations described are indirect substantiation. The results presented here lend further confirmation to those previously reported by us.[1, 2]

The work of Clerc, Paris and Sterne[2] seemed to offer a further possibility of obtaining positive responses in animals suitably treated. Consequently, a saturated solution of octanol in 33 per cent ethyl alcohol was injected intravenously (1.7 c.c. per kg. of body weight) two minutes before the last antigen injection. In addition to a marked narcotic effect there were some slight general but no local reactions. When the octanol was injected ten minutes after the antigen, the results were similar.

This investigation is being continued in an effort to perfect a method which will induce local cerebral anaphylaxis in the dog at will.

SUMMARY

1. Dogs having normal horse serum or fresh egg white in contact with one side of the brain either by means of collodion sacs or by direct injection, reacted focally *on the opposite side of the body* following an intravenous injection of the appropriate antigen. General reactions were also observed. Control animals failed to respond.

2. A number of dogs subjected to the brain operations described, failed to react to the first intravenous injection of antigen. However, upon repeated injections of the same antigen, contralateral focal (as well as general) reactions were elicited. This phenomenon was interpreted as the result of a selective adsorption of antigen in the traumatized brain tissue causing sensitization.

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MONOPHYLETIC SCHEME OF BLOOD CELL FORMATION FOR CLINICAL AND LABORATORY REFERENCE*

KATSUJI KATO, PH.D., M.D., CHICAGO, ILL.

THE origin, development and interrelationships of the formed elements of the blood have furnished a fertile soil for many controversies among anatomists and pathologists. The average clinician, however, has not given much thought to this aspect of laboratory medicine, simply because of an almost unsurmountable degree of confusion existing among the hematologists. Yet it cannot be denied that a practical knowledge of hemocytogenesis is essential to the clinicians, not only for diagnosis of various types of blood dyscrasias, but for a proper evaluation of other pathologic processes as reflected in the blood. It is, of course, a matter of personal choice as to just which theory a clinician should follow. But it is important that a workable theory be chosen, and in order to use it intelligently for clinical purposes, it must be diligently adhered to.

Since the various theories of blood cell formation have been reviewed from time to time, it is not necessary to repeat the task in detail here. Broadly speaking, all schools of hematologic thought may be classified under either monophyletism or polyphyletism. The monophyletic view includes both the so-called extreme unitarians (Dominici, Weidenreich, Dantschakoff, Maximow, Jolly), and the neounitarians (Pappenheim, Ferrata, Downey). The polyphyletic school consists of the dualists (Ehrlich, Nägeli, Schridde, Piney, and Helly), and the trialists (Schilling, Rosenthal), and the extreme polyphyletists (Sabin and her coworkers). The grounds on which these various theories are based are chiefly of academic interest. But from a practical clinical point of view, the monophyletic theory is by far the most satisfactory.

The scheme of the formation and interrelationships of the blood cells here delineated has been found useful as a ready reference in the laboratory and classroom. Monophyletic in principle, its general form has been suggested by a somewhat similar scheme devised by Epstein and published by Downey. All the blood cells illustrated in the chart have been seen by me either in the peripheral blood or in sections of the hemopoietic organs. Their positions are arranged in developmental sequences, and the following explanatory remarks attempt to redefine and properly classify the existing complexity of hematologic nomenclature.

This scheme is made up of five concentric circular zones, numbered by Arabic numerals *1* to *5*, which represent principal stages of maturation of various cell types. Only those stages that are clinically useful in making a satisfactory classification of the cells in the differential count have been illustrated.

*From the Department of Pediatrics, The University of Chicago.
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The central circle contains the generally recognized parent cell of all types of the blood cells, while in the outermost zone are grouped all the mature cells found in the peripheral circulation of normal human subjects. The intermediate zones comprise the cells normally found only in the hemopoietic organs, which, under abnormal conditions, may appear in the peripheral blood. Generally speaking, the more pronounced the degree of abnormal stimulation of the hemopoietic tissues, the greater the number and types of the immature cells which appear in the blood stream. This is best exemplified by the greater percentage of the myeloblasts seen in acute myelogenous leucemia in contrast to the chronic stage in which the myeloblasts are comparatively few.

The radiating lines, starting from the second and third zones and extending to the peripheral zone, divide the blood cells into nine distinct and specific groups, numbered clockwise by Roman numerals, *I* to *IX*. The fact that these radiating lines do not traverse the first and second zones indicates that the cells in these zones have general relationships to all specific cell groups. This discussion follows the various cell series of each sector through the zone sequences in numerical order. These illustrations are based on the actual appearance of the cells in the blood smears made by the method previously described by me and prepared by the panoptic combination method of Pappenheim (May Grünwald stain, followed by dilute Giemsa solution). It may be stated that the Wright stain, when properly applied, also brings out all the characteristic details here described. The names of all cells illustrated in the scheme are given in the Genealogy of Hemocytes accompanying the chart (Table I).

The precursor of all types of blood cells is the undifferentiated mesenchymal cell of the embryo (Zone 1) corresponding in the adult organism to the resting reticulo-endothelial cell, a fixed tissue cell which is not a hemocyte. This cell, however, possesses unlimited mesenchymal developmental potencies, differentiating under normal conditions into the myeloblast, the first general stem cell of the blood. The regeneration of the blood cells beyond the myeloblast stage normally proceeds according to a homoplastic principle. It is only under extremely pathologic conditions that heteroplastic proliferation occurs.

The term myeloblast, according to the unitarian theory here adopted, is applied to the first hemocyte formed by the differentiation of the reticulo-endothelial cell. It is a general hemoblast, a common parent to all the nine specific types of blood cell. In the chart, the five myeloblasts shown in the second zone are all morphologically identical, characterized chiefly by the peculiarly constant pattern of the nuclei. The deeply purple chromatin granules of the myeloblast nucleus are very fine and uniformly distributed. The nucleus itself is relatively large, occupying the major area of the entire cell. There are usually visible nucleoli, varying in number from two to ten. The cytoplasm, scant in amount, usually stains muddy blue, particularly at the periphery. Immediately surrounding the nucleus, there may appear a narrow, slightly creamy, hyaline-clear zone. The bluish-stained cytoplasmic rim often appears granular, especially when seen in company with granulocytes (as in myelogenous leucemia) and erythroblasts (as in erythroblastosis).

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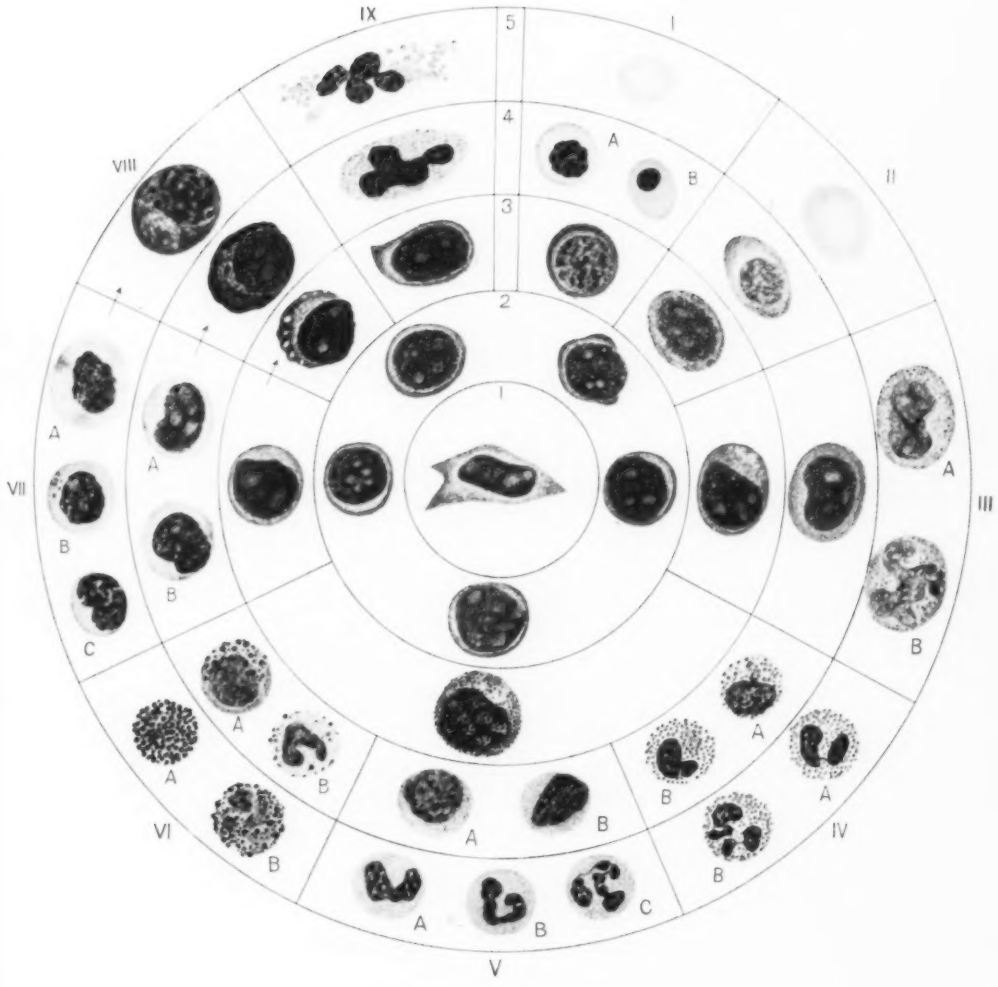

CHART I.

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Sometimes, however, as seen in lymphatic leucemia, this cytoplasmic ring may appear homogenous. The myeloblast thus described is morphologically identical with the myeloblast of Nägeli, Downey, and Piney. In the literature, it is synonymously known as the lymphoidocyte (Pappenheim), the indifferent lymphoid cell of the marrow (Michaelis and Wolff), and the hemocytoblast (Ferrata). Maximow, however, was unable to distinguish the lymphocyte and the myeloblast on the basis of their cytologic structures, and his hemocytoblast is not the same cell as the myeloblast here described.

Sectors I and II depict the cells of the erythrocytic series. It is now generally agreed that the cells of the normoblastic series represent the definitive or permanent erythrocytes in the normal adult organism, replacing the primitive or embryonic erythrocytes of the megaloblastic group. These two series of erythrocytes, therefore, are distinct not only in their cytologic structures,

TABLE I

GENEALOGY OF HEMOCYTES

ZONE 1 PARENT CELL	ZONE 2 GENERAL HEMOBLAST	ZONE 3 SPECIFIC HEMOBLASTS	ZONE 4 INTERMEDIATE HEMOCYTES	ZONE 5 MATURE HEMOCYTES OF CIRCULATION	SECTOR
Undifferentiated mesenchymal cell (embryo) or Reticuloendothelial cell (adult)	Myeloblast	Pronormoblast (Proerythroblast)	A. Polychromatic normoblast B. Orthochromatic normoblast	Orthochromatic erythrocyte	Sector I
		Promegaloblast	Megaloblast	Megalocyte	Sector II
		Monoblast	Promonocyte	A – B Monocytes	Sector III
		Leucoblast	A. Eosinophilic myelocyte B. Eosinophilic metamyelocyte	A – B Eosinophilic polymorphonuclears	Sector IV
			A. Neutrophilic promyelocyte B. Neutrophilic myelocyte	A. Neutrophilic metamyelocyte B. Neutrophilic staff cell C. Neutrophilic polymorphonuclears	Sector V
			A. Basophilic myelocyte B. Basophilic metamyelocyte	A – B Basophilic polymorphonuclears	Sector VI
		Lymphoblast	A – B Prolymphocytes	A. Large lymphocyte B. Medium lymphocyte C. Small lymphocyte	Sector VII
		Plasmoblast (Lymphoblastic plasma cell)	Proplasmocyte	Plasmocyte (Plasma cell)	Sector VIII
		Megakaryoblast	Megakaryocyte	Thrombocytes (platelets) and nuclear fragments of megakaryocyte	Sector IX

but also in their functional significance. In the clinical literature, the megaloblast is frequently referred to as representing the most immature stage in the development of the normoblastic erythrocytes, but such a view is the result of confusing the phylogenetic position of the megaloblast with the ontogeny of the normoblast.

In the normocytic series, the most primitive cell is the proerythroblast (Sector I, Zone 3), a cell referred to in the literature also as the basophile erythroblast (Ferrata) or the macroblast (Naegeli). This cell corresponds to the promegaloblast in the degree of maturity, their chief difference being their characteristic nuclear pattern. The chromatin granules in the pronormoblast, show a greater tendency to aggregation than in the promegaloblast, clumping of chromatin in the former cell already suggesting a cart wheel arrangement. The cytoplasm, deeply blue and somewhat granular, appears much more condensed and irregular in outline than in the promegaloblast. The presence of hemoglobin is not yet detectable.

The early normoblast (Sector I, Zone 4, *A*) being a more mature nucleated red blood cell, definitely demonstrates the presence of hemoglobin. The nuclear chromatin is characteristically arranged in cart wheel shape, though the cytoplasm may be still polychromatophilic. The nuclear membrane is usually heavy and distinctly visible. In a later stage, this polychromasia of the cytoplasm is almost completely replaced by pure hemoglobin, but with a greatly condensed nucleus (Sector I, Zone 4, *B*). The cell is then called orthochromatic normoblast. This latter nucleus is finally lost by extrusion, with the resulting mature erythrocyte (Sector I, Zone 5). Such terms as the orthonormocyte, the macronormocyte, and the micronormocyte, used by some hematologists, are all mature erythrocytes of this series, differentiated on the basis of their cytologic dimensions.

The cells of the megaloblastic group are illustrated in Sector II. The first distinct cell of this series, called promegaloblast (Sector II, Zone 3), is characterized by somewhat coarse chromatin granules, uniformly distributed throughout the nucleus, and with very little tendency to form into aggregations. The nuclear membrane is indistinct and the nucleolus sometimes visible. The parachromatin spaces are much more conspicuous than in the stem cell, and the cytoplasm, still very dark blue and rather granular. As a rule, this promegaloblast is much larger in dimensions than the comparable cell of the normoblast series, the pronormoblast. Like the latter cell, the promegaloblast shows no evidence of hemoglobin in its protoplasm.

The megaloblast is shown in the fourth zone of Sector II. This cell is characterized by its huge size, and by its primitive nuclear pattern, which, however, is beginning to show some evidence of chromatin condensation, causing the nucleus to appear coarsely reticular. The nuclear membrane is usually very indistinct. The most characteristic aspect of this cell is the fairly abundant presence of hemoglobin, in sharp contrast to the cells of similar nuclear immaturity in the normoblastic series. The slightly polychromatophilic cytoplasm usually presents granular appearance, especially near the periphery of

the cell. The final mature cell of the megaloblastic group is the megalocyte (Sector II, Zone 5) which, after having passed the various stages of nuclear condensation, has completely cast out its nucleus.

The normal formation and regeneration of the red blood cells in the adult organism involve only the normoblastic series, but under pathologic conditions the megaloblastic group may be stimulated. Clinically there are only a very few conditions in which such stimulation is reflected in the blood stream, chief among which are pernicious anemia, intestinal parasites, calcinomatosis and severe types of erythroblastosis in infants. In fetal hemopoiesis, the megaloblastic form of regeneration is said to be a normal function and such a phenomenon may be found occasionally and only temporarily in postnatal life of prematurely born infants.

Sector III contains the cells of the monocytic series, of which the monoblast (Zone 3) is the most primitive. The monoblast is characterized by a slight tendency of the chromatin granules to become reticular. The nucleoli are definitely visible. The granular cytoplasm is still deeply basophilic, though usually not as marked as that of the proerythroblast or the promegaloblast. The cell in the fourth zone of Sector III is the promonocyte, characterized by a still further condensation of reticular chromatin strands, with a frequently visible nucleolus. Its cytoplasm is basophilic, less pronounced than that of the monoblast, and shows a greater degree of granular appearance. Fine azur granules are only occasionally seen, being frequently absent altogether. This type of cell is occasionally seen in the peripheral circulation under conditions of excessive stimulation of the reticulo-endothelial system. The mature monocytes of the peripheral blood (Sector III, Zone 5, *A* and *B*) are characterized by the pale bluish granules in the cytoplasm and an abundance of nonspecific fine azur granules, often spoken of as azur dust. Their nuclear pattern is highly reticular in its chromatin arrangement. The configurations of the nuclei are greatly varied, sometimes showing a kidney-beanlike indentation on one side, in the deep crevice of which upon close examination are usually seen two small projections; or again the nuclei may be greatly elongated, folding and bending their extended nuclear processes upon one another, producing fantastic shapes of infinite varieties. This latter type of monocyte is sometimes referred to as a Rieder cell.

Sectors IV, V, and VI illustrate the three recognized varieties of specific granulocytes, of which the leucoblast (Pappenheim) is the common precursor, the first distinct and specific stem cell differentiating in the granulocytic direction from the myeloblast, its general stem cell. The leucoblast (Sectors IV, V, VI, Zone 3) is characterized by its large size and slightly reticular nucleus with distinctly visible nucleoli. Its nuclear membrane is usually invisible and cytoplasm moderately blue in staining though lighter than the blue of the myeloblast. The most distinguishing feature, however, of the leucoblast is the abundance of nonspecific azur granules, scattered profusely over the surface of the entire cell, ranging in number from about twenty to sometimes one hundred or more. It is from this common specific hemoblast

that arise the specifically granulated promyelocytes, myelocytes, metamyelocytes, and finally mature polymorphonuclear leucocytes.

In Sector IV, Zone 4, are shown the eosinophilic myelocyte (*A*), and metamyelocyte (*B*), which develop into mature eosinophilic granulocytes with segmented nuclei (Sector IV, Zone 5, *A* and *B*). In the eosinophilic myelocyte, the specific granules do not always appear typically red, but rather dark grayish, due to the immature state of some granules. The nucleus of the mature eosinophilic granulocyte usually is made up of two elongated segments, resembling a pair of butterfly wings, but in pathologic conditions, it shows a greater number of segments.

The neutrophilic promyelocyte (Sector V, Zone 3, *A*), characterized by the coexistence of both the primitive basophilic cytoplasm and the specific neutrophilic granules, is the precursor of the neutrophilic myelocyte. In the latter cell (Sector V, Zone 3, *B*) the entire cytoplasm is converted into mature specific neutrophilic granules. The nuclei of both cells are still undifferentiated, but the chromatin aggregation is pronounced.

Sector V, Zone 5, *A*, *B*, and *C* illustrates the three degrees of mature neutrophilic granulocytes in normal circulation, respectively designated as the metamyelocyte, the staff neutrophile and the segmented neutrophile, in order of maturity. The youngest of these, the metamyelocytes, is found normally only in the blood of the newborn infants during the first few days of postnatal life. The staff cells characterized by a further differentiation of the nucleus, but not yet showing its segmentation, are also normally present in the peripheral blood, their increase indicating a correspondingly good prognosis in cases of acute infection. The typical segmentation of the nucleus, as in mature granulocytes, must always show the presence of two or more segments of the nuclear substance, mutually connected by a very thin filament of the same substance. Occasionally nuclear buds may be seen projecting from any portion of the nuclear lobules. As the granulocyte matures, the number of segments increases, and in certain stages as many as ten or twelve lobules may be distinguished. The so-called qualitative differential count of the Arneth hemogram is based on the number of segments thus demonstrated. Occasionally one or two light bluish protoplasmic granules of varying sizes may be found in the cytoplasm of segmented neutrophilic granulocytes. They have been called Döhle's inclusion bodies, but seem to possess no clinical significance.

The specific basophilic myelocyte (Sector VI, Zone 4, *A*) possesses a rather grayish blue cytoplasm with a rounded configuration of the nucleus containing characteristically clumped chromatin granules. The morphology of the basophilic metamyelocyte (Sector VI, Zone 4, *B*) is exactly identical with that of the myelocyte, except the presence of one deep indentation of the nucleus on one side. The specific granules may be either scant or abundant, though never as plentiful as the granules of the mature basophilic granulocyte, the nucleus of which is segmented (Sector VI, Zone 5, *A* and *B*). The basophilic granulocyte is synonymously known as the mast cell in the older literature.

The cells of the lymphocytic series are shown in Sector VII, the most primitive being the lymphoblast (Zone 3) which develops from the myeloblast

as may be traced in the blood of subacute and acute lymphatic leucemia. This lymphoblast is the specific stem cell for all types of lymphocytes. In general structure the cell is definitely lymphocytic, in that the chromatin granules are much coarser than in its parent cell, the myeloblast. The cytoplasm becomes a little more abundant as compared with the myeloblast, and the nucleoli, usually two in number, are distinctly visible. The more mature cells of this series are the large and small prolymphocytes, morphologically identical (Sector VII, Zone 4, *A and B*). Their nuclei contain definitely characteristic chromatin aggregations with occasionally visible single nucleoli. Their cytoplasm, peculiarly hyaline, stains sky-blue with no protoplasmic granulations. From either these large or these small prolymphocytes, the three recognized varieties of mature lymphocytes develop, but the exact antecedent of each has not been clearly established. The large lymphocyte (Sector VII, Zone 5, *A*) has characteristic aggregation of chromatin into heavily staining bars and clumps. The cytoplasm, hyaline, transparent and homogeneous, often displays a tendency toward irregular protoplasmic distribution of blue staining. Occupying an intermediate position among the mature lymphocytes, the medium lymphocyte or mesolymphocyte (Sector VII, Zone 5, *B*) is identical with the large type in morphologic details, except in the occasional presence of visible nonspecific azur granules much coarser than those seen in the monocytes. These granules are practically never seen in abundance and occur at one portion of the hyaline cytoplasm usually near the nuclear indentation. The small lymphocyte (Sector VII, Zone 5, *C*), characterized by a very small amount of cytoplasm, is frequently more definitely condensed as to nuclear chromatin than the two lymphocytes above described.

The lymphocytes have chiefly been the center of many hematologic controversies, because of their developmental potencies exhibited under pathologic conditions. Some unitarians believe that the mature lymphocytes of the normal blood, particularly the small variety, may dedifferentiate under abnormal stimulation back into the myeloblast, through the lymphoblast stage. This myeloblast may in turn redifferentiate into any type of hemocyte. Thus the blood picture reported by Logefeil under the title of mixed leucemia may be interpreted to mean only a certain stage of myeloid leucemia, in which the lymphocytes are dedifferentiating back into the myeloblast and then finally into the granulocytes.

In Sector VIII are shown three varieties of hemocytes usually called plasma cells (Cajal, Unna, Marschalko, Schridde). The typical plasma cells are present in the normal blood in a small percentage and, therefore, their occurrence has no pathologic implications. The usual type, here called plasmocyte (Zone 5), is characterized by its ultramarine blue, opaque cytoplasm, somewhat granular, a nucleus often eccentrically placed, and a chromatin aggregation usually in cart wheel arrangement. Also, just adjacent to the nucleus, appears a narrow clear zone sharply contrasting with the opaquely blue cytoplasm. The plasma cell with a typical cytoplasm but with an immature nuclear pattern has been recognized by some observers to belong to the plasmocytic series. Such a cell has been called lymphoblastic plasma cell

(Schridde), and it is derived directly from the lymphoblast. In the present scheme, this variety of plasma cell is placed in Sector VIII, Zone 3, and tentatively called plasmoblast. It is conceivable that this apparently primitive cell may give rise to a more typical plasma cell. The so-called irritation form of Türk, according to some investigators, may be identical with the lymphoblastic plasma cell, as its nuclear pattern is found to be immature. When it occurs in myelogenous leucemia, it is undoubtedly derived from the myeloblast. Again, a cell which appears to possess a nuclear chromatin arrangement of intermediate maturity, here called proplasmocyte (Sector VIII, Zone 4), may be found in the organs of such conditions as plasma cell leucemia (lymphoadenosis leucemica plasmacellularis, Piney) and plasmacytoma or plasma cell myeloma. Occasionally, the plasma cell may show, in its cytoplasm, the presence of nonspecific azur granules (plasmacytoid cell of Piney). Vacuoles are also frequently noted in the cytoplasm of all types of plasma cells.

The cells of the plasmocytic group offer the greatest difficulty in establishing their respective positions in the developmental scale. The genetic arrangement here proposed is merely an attempt to classify the cells of this group solely on the basis of chromatin immaturity. It does not, therefore, necessarily represent the cytogenetic sequence. The histologic evidence at present available points very strongly to the lymphocytic origin of most of the plasmocytes (Marschalko, Krompecher, Naegeli, Jolly, Maximow, Downey), although there are a number of investigators who hold a histogenous origin from connective tissue cells such as clasmatocytes, resting wandering cells, and adventitial cells (Unna, Cajal, Pappenheim, Marchand, Foa, Türk). Again, some observers believe that the plasma cells originate from immature blood cells, such as myeloblasts and erythroblasts through aberration and abortion (McGowan, Jordan, Dawson and Masur). While most plasma cells seem to be derived from mature lymphocytes, there is much evidence to indicate that all of the cell types enumerated above may transform to plasma cells which, however, do not necessarily acquire the cart wheel nucleus. This indicates that plasma cell formation is a reaction which may involve several types of cells, more or less closely related among themselves (Downey), and may include even the fibroblasts (Unna).

The various stages in the development of the megakaryocyte and platelets are traced in Sector IX. Most primitive is the megakaryoblast (Sector IX, Zone 3). Surrounded by a definitely hyaline zone and containing distinctly visible nucleoli, its nucleus is marked by a somewhat densely arranged reticular chromatin pattern. The cytoplasm is slate gray. This cell develops into the typical megakaryocyte (Sector IX, Zone 4), a mature cell of huge dimensions, with a characteristic multilobulated large nucleus, taking an extremely deep stain. The cytoplasm, hyaline in ground substance, contains numerous fine purplish-stained granules. Portions of the cytoplasm may be seen breaking off as individual platelets, here called thrombocytes. Normally the megakaryocyte is found in the bone marrow of adult subjects and also in the hepatic sinusoids of embryonic organs. The typical thrombocyte consists of two distinct parts: the chromomere which is highly refractile and composed

of purple granules, and the hyalomere, taking on a pale blue stain, homogeneous in appearance and extremely flexible. The individual platelets vary greatly in size, the average being 3 micra in diameter. They tend to agglutinate with one another, forming irregular islands on the smear. Frequently large masses, possessing every property of a typical platelet, are found. The thrombocytes are normal constituents of the peripheral blood, and occasionally even fragments of megakaryocytic nucleus may be found under normal conditions. Pathologically the platelets may be greatly reduced (as in thrombocytopenic purpura) or much increased (as in posttraumatic hemorrhage and myelogenous leucemia).

It is, in conclusion, to be emphasized that the foregoing conception is not an attempt to formulate any new theory of hemocytogenesis. Rather it has endeavored only to: (1) systematize the hematologic nomenclature; (2) to redefine the various cell types (chosen wholly on the basis of clinical significance); and (3) to arrange them in proper genetic relationship according to the monophyletic (neounitarian) theory.

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SOME OBSERVATIONS ON THE COMPARATIVE EFFECTIVENESS OF MERCURIAL DIURETICS WITH AND WITHOUT THEOPHYLLINE (MERCUPURIN, SALYRGAN, ETC.)*

MARSHALL N. FULTON, M.D., BOSTON, MASS., AND A. HUGHES BRYAN, M.D., CHICAGO, ILL.

BECAUSE of their marked diuretic effect, generally superior to that of xanthine compounds, the mercurial diuretics now occupy an important place in the treatment of patients with edema. The purpose of this communication is to report our early experiences with two new mercurial diuretics. (1) mercupurin, which contains theophylline both free and chemically bound with a complex organic mercury compound, structurally quite different from salyrgan, and (2) a combination of a mercurial diuretic to which free theophylline has been added in a concentration of 5 per cent. The latter combination, which is not available commercially, will be referred to in this paper as diuretic X.

The first of the mercurial diuretics to attain common use, merbaphen or novasurol, was found to cause toxic manifestations in patients. Though excellent in its diuretic effect, its continued use frequently led to such symptoms as salivation, gingivitis, colitis, and proctitis.[1] Johnstone and Keith[2] found that in rabbits it produced renal lesions identical with those caused by mercurial compounds in common use, although its toxicity appeared to be less than that of mercuric chloride when based on the amount of pure mercury contained in the two.

Mersalyl or salyrgan was introduced in this country about 1927. Although containing a slightly higher percentage of mercury than novasurol (39 per cent compared with 33 per cent), it was found to be much less toxic to experimental animals and to man, and to have as satisfactory a diuretic effect, if indeed not a better one, than novasurol. It has been suggested[3, 4] that the difference in toxicity of the various mercurial compounds depends on

*From the Medical Clinic, Peter Bent Brigham Hospital, and The Department of Medicine, Harvard Medical School.

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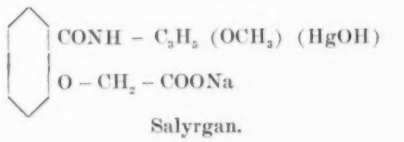
the chemical linkage of the mercury with the rest of the molecule rather than the actual percentage present. Though isolated reports of ill effects attributed to salyrgan have appeared in the literature,[5, 6] the absence of toxic effects following its use has been noted repeatedly. Several reports have appeared[7, 8] of patients to whom salyrgan has been administered over a period of five years at six- to seven-day intervals without causing any untoward symptoms or without diminution of its effectiveness. Tarr and Jacobson[1] encountered only two instances of mild stomatitis in a group of patients receiving more than 3,000 injections of this drug. In thirty autopsies on patients who had been given from 3 to 130 c.c. of salyrgan, they found only one instance in which the kidneys showed a lesion suggestive of injury from mercury. In this laboratory a daily injection for thirty days of 1 c.c. of salyrgan intravenously into a dog weighing eighteen kilos produced no untoward symptoms, and the kidneys at autopsy showed no abnormality microscopically. The chief disadvantages to the use of salyrgan are (1) its ineffectiveness by the oral route, (2) its tendency to cause venous thrombosis and occasionally necrotic skin lesions at the site of injection, and (3) the pain which it causes on intramuscular injection. However, because of its excellent diuretic effect and the absence of significant toxic sequellae following its repeated administration, salyrgan has become at present a most widely used and popular diuretic drug.

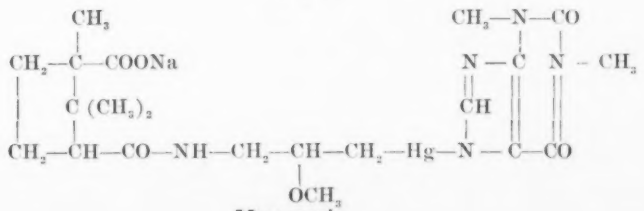
Is a more ideal mercurial diuretic available?

A number of complex organic mercury salts have been tried out from time to time in the hope of securing, if possible, other compounds of low toxicity which have a pronounced diuretic effect. In 1928 v. Issekutz and v. Vegh[9] reported on the comparative value of a group of mercurial compounds, studying the diuretic action of these substances in rabbits and their toxicity in rats. They found that their preparation No. VI, when combined with theophylline, yielded the best results. In this form it increased the urine flow in rabbits considerably more than either salyrgan or novasurol and was relatively nontoxic.

A combination similar to, if not identical with, this last drug has been in use for several years in European clinics under the name of novurit and has been introduced recently in this country as mercupurin. Chemically this substance is distinctly different from salyrgan as shown by the formulas:



Salyrgan.



Mercupurin.

The mercurial salt of mercupurin contains 41 per cent mercury. An important difference between salyrgan and mercupurin is that the mercurial salt of the latter has bound with it chemically 3.5 per cent of theophylline. To this is added 1.5 per cent of free theophylline. Reports in the European literature vary as to the effectiveness of this drug in comparison with salyrgan and novasurol. Thus, some authors have found it at times more effective,[10, 11] while others have reported no marked difference.[12, 3] It has been given repeatedly both intravenously and intramuscularly without causing toxic effects. Hahn[3] reported freedom from pain in patients following intramuscular injection of novurit (mercupurin) and a greater diuresis than with salyrgan during the first four hours. The twenty-four-hour output, however, was not essentially different with the two drugs. Crawford[15] has studied recently the comparative effectiveness of salyrgan and mercupurin in a group of edematous patients, finding mercupurin the more efficacious of the two in the majority of cases, though not in all.

DeGraff and others[16] found in cardiac patients that the diuretic effect of mercupurin is greater than that of mercurial diuretics not containing theophylline. They attribute this superiority chiefly to the presence of theophylline.

The other preparation used in this study, diuretic X, was a recognized mercurial diuretic to which was added theophylline in the strength of 5 per cent, an amount comparable to that contained in mercupurin.

Certain considerations favor the addition of a xanthine compound to a mercurial diuretic as in mercupurin and diuretic X. In the first place the contention is advanced by some that these two types of drugs have different modes of action, the mercurial diuretic affecting chiefly the storage of water in the tissues and the other acting directly on the kidneys. Second, investigators, who believe that both types affect the kidneys predominantly, have presented evidence suggesting that salyrgan acts by decreasing tubular reabsorption, whereas theophylline causes diuresis by increasing glomerular filtration. If either of these arguments is valid, a combination of the two drugs might yield a preparation of considerable diuretic potency. Thus Herrmann and others[13] have advocated the administration of salyrgan along with various xanthine diuretics to obtain a maximum urine output in patients with cardiac edema. Last, v. Issekutz and v. Vegh[9] felt that such a preparation should be less painful to patients when injected intramuscularly than solutions of the mercurial compounds alone, because the weakly acidic properties of theophylline would neutralize the alkaline reaction resulting from the hydrolysis of the complex mercurial salt.

During the past year we have observed the action of these two theophylline-containing diuretic mixtures, comparing them with salyrgan in hospitalized patients and experimental animals. We have been particularly interested in their diuretic potency, in the pain which they occasion when injected intramuscularly into patients, and in the skin reaction following their injection subcutaneously into animals. The results of this study are presented below.*

*We wish to express our thanks to the Campbell Products, Inc., 79 Madison Avenue, New York, N. Y., for the mercupurin and to the Winthrop Chemical Co., Inc., 170 Varick Street, New York, N. Y., for the salyrgan used in these studies.

OBSERVATIONS IN PATIENTS

1. *Mercupurin and Salyrgan.*—The plan of observation with patients, all of whom had edema due to heart failure, was to alternate the diuretics at intervals of three to five days. The subjects were observed first for several days for any evidence of spontaneous diuresis or of that due to digitalis before the diuretics were given. All of them received digitalis as their clinical condition indicated during their stay in the hospital, and the majority received 1.0 gm. of ammonium chloride three to four times daily. Not all patients of this type are entirely satisfactory for determining the comparative effectiveness of different diuretics. The most satisfactory measurements are to be had in those with marked or chronic edema who require not two or three but a dozen good diureses before the edema disappears. Even if a carefully controlled régime with measured and evenly distributed intake of food and fluid is carried out, the extent of the edema will influence considerably the response to a diuretic. Frequently the patient may have a brisk diuresis the first time a diuretic is given and will lose sufficient edema so that the same dose of the same drug given several days later will not induce a comparable diuresis. Last, to avoid presenting a mass of data we have tabulated the average urine output for a given dose of a drug when that drug was given more than once to a patient. Such a method of tabulation is open to criticism, for in instances where a marked diuresis is followed by a relatively slight one, the average might be conspicuously lowered by the second result. All of these facts must be considered in evaluating the data at hand.

The results are presented in Table I. It will be noticed that there are specific instances in which mercupurin caused a significantly greater urine output than salyrgan, while in others the opposite was the case. The general impression created during the time these observations were made was that the two preparations were about equally efficacious in causing diuresis. Neither one produced any definite signs of kidney damage, as judged by the urine, by phthalein excretion or by nitrogen retention, nor did either one lead to other signs of mercurial intoxication. The few times that mercupurin was given intramuscularly it caused pain which seemed to be of no less degree, though shorter in duration, than that usually caused by salyrgan. The diuretic response to intramuscular injection was adequate though somewhat less than that following administration by the intravenous route.

2. *Diuretic X and Salyrgan.*—In the study of diuretic X in this clinic more attention was paid to determining the amount of pain it caused when injected intramuscularly than to a comparison of its diuretic action with that of salyrgan. Inasmuch as this preparation did not cause a slough in dogs and rabbits, when injected intra- or subcutaneously (see below), it was thought that intramuscular injection might be a painless procedure. This was found not to be the case. The patients complained on the whole about as much from the discomfort following diuretic X as from that following salyrgan intramuscularly. In the patients in whom the diuretic response of the two drugs could be compared, no greater diuresis was evident from the presence of theophyl-

line in diuretic X (Table II). It was our impression that the responses to diuretic X were in no way superior to those obtained with salyrgan.

OBSERVATIONS IN ANIMALS

The comparative action of the three diuretics was measured in normal dogs and rabbits by administering these preparations to the animals at intervals

TABLE I

AVERAGE DIURESES FOLLOWING MERCUPURIN AND SALYRGAN IN SIXTEEN PATIENTS WITH EDEMA OF CARDIAC FAILURE

MERCUPURIN				SALYRGAN			
PATIENT	NO. OF OBSERVATIONS	AVER. 24-HOUR FLUID INTAKE C.C.	AVER. 24-HOUR URINE OUTPUT C.C.	NO. OF OBSERVATIONS	AVER. 24-HOUR FLUID INTAKE C.C.	AVER. 24-HOUR URINE OUTPUT C.C.	DOSE INTRAVENOUSLY
1	5	710	3,915	4	790	3,215	1 c.c.
2	2	1,150	2,600	3	1,100	3,250	1 c.c.
	3	1,000	5,220	3	950	2,900	1.8 c.c.
3	4	1,175	6,935	4	1,165	6,155	1 c.c.
	3	1,000	6,590	4	1,000	4,750	2 c.c.
4	3	1,170	4,530	4	1,080	4,450	1 c.c.
5	2	1,200	2,350	4	1,150	2,680	1 c.c.
6	1	–	4,000	2	–	3,000	1 c.c.
	2	–	4,625	3	–	4,660	2 c.c.
7	1	1,500	3,575	2	1,100	3,300	1 c.c.
	1	1,000	5,400	1	1,050	3,250	2 c.c.
8	1	1,900	7,110	3	1,500	3,130	1 c.c.
9	1	2,000	5,300	2	1,700	4,475	1 c.c.
10	3	1,060	2,140	2	1,150	2,600	1 c.c.
	1	1,200	3,500	2	1,250	1,950	2 c.c.
11	1	1,000	1,800	1*	900	2,720	1 c.c.
12	1	1,600	4,400	1*	1,000	4,770	1 c.c.
13	1	700	4,400	1*	1,100	4,950	1 c.c.
14	1	1,000	2,400	1*	1,200	1,400	1 c.c.
15	1*	1,500	2,450	1	1,450	1,550	1 c.c.
16	1	1,250	3,950	1*	1,500	3,300	1 c.c.

*Indicates drug given first.

TABLE II

AVERAGE DIURESES FOLLOWING DIURETIC X AND SALYGRAN IN SIX PATIENTS WITH EDEMA OF CARDIAC FAILURE

DIURETIC X				SALYRGAN			
PATIENT	NO. OF OBSERVATIONS	AVER. 24-HOUR FLUID INTAKE C.C.	AVER. 24-HOUR URINE OUTPUT C.C.	NO. OF OBSERVATIONS	AVER. 24-HOUR FLUID INTAKE C.C.	AVER. 24-HOUR URINE OUTPUT C.C.	DOSE
1	1*	750	1,950	1	700	2,000	1 c.c. i.m.
2	1	1,300	1,400	1*	1,800	3,000	1 c.c. i.v.
3	1*	1,200	2,700	1	1,200	2,400	2 c.c. i.m.
4	1*	1,100	3,500	2	1,350	1,950	2 c.c. i.m.
5	1	450	1,300	2*	1,100	2,300	2 c.c. i.m.
6	1*	1,100	1,300	1	600	1,750	1 c.c. i.m.

*Indicates drug given first.

of three to seven days. The animals were kept on a constant diet and were allowed water *ad libitum* until two hours before the diuretic was given. Food was withheld on the day that the measurements were made. The urine was collected in metabolism cages during a six-hour period. The diuretic was given at

the end of the second hour so that its effect was observed for four hours, at the end of which time the bladder was emptied by catheter. As a control, the same procedure was carried out on other days without administering a diuretic. This method affords a satisfactory, though somewhat rough, basis for comparing the effects of diuretics when given repeatedly to the same animals. The urine was measured as to volume and chloride content. To obviate chloride depletion, which will occur from repeated drug-induced diureses, an amount of sodium chloride containing slightly more chloride than that excreted in the urine was administered by stomach tube following the six-hour period of urine collection.

The results are shown in Table III. It was found in rabbits that mercupurin increased the urine volume 3 to 3½ times that observed in control

TABLE III

URINE AND CHLORIDE OUTPUT IN RABBITS AND DOGS FOR A SIX-HOUR PERIOD DURING FASTING STATE

NO. OF EXPERIMENTS	NO. OF ANIMALS	AVERAGE URINE VOLUME C.C.	AVERAGE URINE CHLORIDE MG.	
		Rabbits		
48	14	18	15.6	Controls
13	9	35	127.0	Salyrgan 0.2 c.c. i.v.
8	4	24	54.0	Salyrgan 0.2 c.c. i.m.
11	8	50	208.0	Mercupurin 0.2 c.c. i.v.
8	4	65	262.0	Mercupurin 0.2 c.c. i.m.
11	8	83	216.0	Diuretic X 0.2 c.c. i.v.
7	5	84	301.0	Diuretic X 0.2 c.c. sub. cut.
7	5	73	284.0	Salyrgan 0.2 c.c. i.v. plus Theophylline 10 mg. sub. cut.
		Dogs		
7	2	64	35.4	Controls
7	2	143	933.0	Salyrgan 0.75 to 0.8 c.c. i.v.
7	2	188	1,656.0	Mercupurin 0.75 to 0.8 c.c. i.v.
9	3	199	1,723.0	Diuretic X 0.75 to 0.8 c.c. i.v.

experiments, and the chloride output 13 to 17 times. Salyrgan was less effective in rabbits, augmenting the urine output only from 18 c.c. to 24 c.c. (when given intramuscularly) and to 35 c.c. (when given intravenously). This difference in the response of rabbits to the two drugs may be due largely, if not entirely, to the presence of theophylline in the mercupurin. Rabbits respond to theophylline with a good diuresis.[14] In fact the largest diuresis observed in these animals occurred with the combination containing free theophylline in the form of diuretic X. With this preparation the urine volume exceeded the control by four to four and one-half times. This occurred following both the intravenous and subcutaneous administration of the drug. Furthermore, when 10 mg. of theophylline (the amount contained in 0.2 c.c. of mercupurin or diuretic X) were given subcutaneously along with 0.2 c.c. of salyrgan intravenously, the resulting diuresis was definitely greater than that following either mercupurin or salyrgan alone. These findings point favorably to the

effectiveness of combining the mercurial diuretics with theophylline. However, the fact that diuretic X did not prove to be superior to salyrgan or mercupurin in human beings emphasizes the inadvisability of judging the value of a drug of this type in human beings solely from its action in rabbits.

In dogs the response to the three preparations is shown in Table III. Here, again, the mercurial diuretics in combination with theophylline produced a greater output of urine and chloride than did salyrgan alone. The urine excretion was three times, the chloride output nearly fifty times that observed in the control experiments, whereas with salyrgan alone the output was increased only 2.2 and 26 times, respectively.

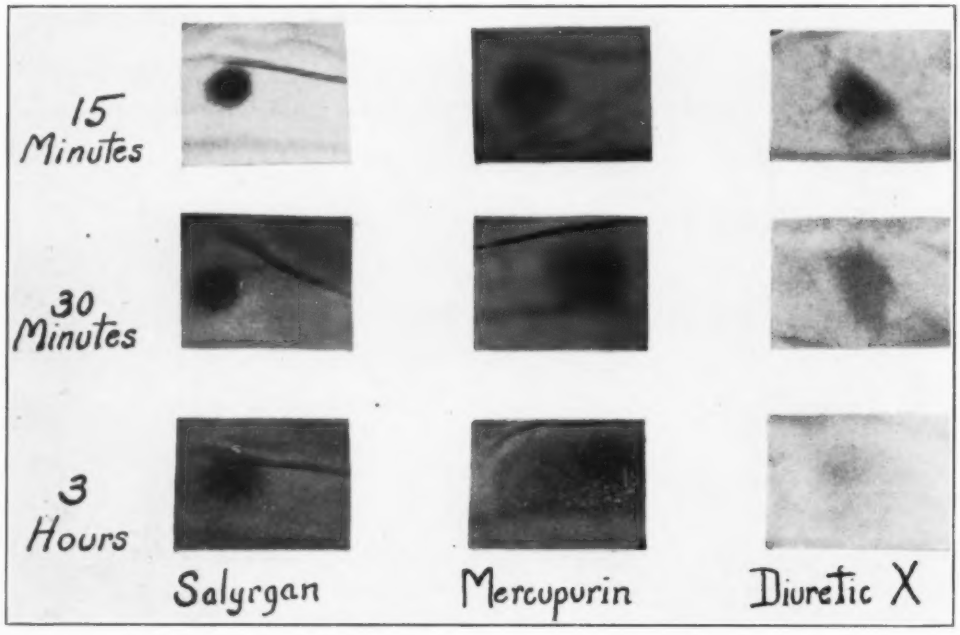


Fig. 1.—X-ray photograph of rabbit's ear at various time intervals following intracutaneous injection of 0.05 c.c. of salyrgan, mercupurin, and diuretic X.

SLOUGHS

One of the untoward events in the use of the mercurial diuretics, as noted above, is the occasional production of lesions at the site of injection due to the accidental leakage of the fluid into the skin. We have studied the effect of salyrgan, mercupurin, and diuretic X when injected intracutaneously in animals in doses of 0.05 to 0.1 c.c. The findings may be summarized by saying that the reaction following salyrgan is somewhat more intense than that produced with mercupurin, although both cause a definite necrotic lesion. With salyrgan there is more commonly sloughing of the overlying skin with a resulting open sore, whereas mercupurin usually produces an indurated lesion with a scab formation in the center. The usual precautions, therefore, that are taken to avoid slough formation in the administration of salyrgan need to be observed in the use of mercupurin.

The localization and disappearance of mercury at the site of injection into the skin of a rabbit's ear can be followed by x-ray photographs. This is illustrated in Fig. 1. Immediately after the injection of salyrgan the lesion casts a sharply outlined shadow which is still plainly visible at the end of three hours. With mercupurin the shadow cast just after injection is more diffuse. It tends to disappear promptly so that in three hours there remains only an ill-defined area of increased density.

There was a very striking absence of slough formation in animals following the subcutaneous injection of diuretic X. This preparation in amounts of 0.05 to 0.1 c.c. was injected into or under the skin forty-one times in five rabbits and several times in dogs. In no instance did a definite slough form. The usual lesion produced was an area of erythema and edema 0.5 to 1.5 cm. in diameter, which disappeared within twenty-four to forty-eight hours. A possible explanation of this apparent "protective" action of theophylline is that offered by v. Issekutz and v. Vegh,[9] who felt that the theophylline neutralized the alkaline reaction produced by the hydrolysis of the complex mercury salts in watery solution. From the x-ray photographs taken following the injection of diuretic X (Fig. 1) it is apparent that this substance is not sharply localized at the site of injection as in salyrgan and, moreover, disappears rapidly, indicating that it is absorbed promptly.

SUMMARY

The purpose of this communication is to report our observations on the effectiveness of two mercurial diuretics containing theophylline compared with salyrgan. The first of these, mercupurin (novurit), contains theophylline in both free and combined form, the usual dose of 1 c.c. supplying approximately 50 mg. of theophylline. As far as can be judged from its use in a group of sixteen hospital patients with edema of cardiac failure, mercupurin is quite as effective as salyrgan in increasing the output of urine. In specific instances it produced a distinctly greater diuresis, possibly due to the added effect of theophylline. In other instances, there was no evident superiority of the one preparation over the other. It is not a new experience to find one diuretic more effective than another in a given individual, and further use of these drugs will doubtless reveal instances in which better results may be obtained with mercupurin than with salyrgan and vice versa. The other preparation, diuretic X, containing theophylline which merely has been added to an active mercurial diuretic, and which is not available commercially, showed no advantages over salyrgan in six patients. All three of these drugs were equally irritating when injected intramuscularly in patients; in animals, those containing theophylline were less irritating.

Both of the theophylline-containing drugs induced a somewhat greater excretion of urine and chloride in rabbits and dogs than did salyrgan. This fact again would appear to be related to the added effect of theophylline which of itself has a diuretic action in these animals. The somewhat greater

effect of mercupurin and diuretic X in animals emphasizes the inadvisability of judging the efficiency of a diuretic drug solely from its action in experimental animals.

Clinically no preference for any one of these three diuretics, salyrgan, mercupurin, and diuretic X, has been demonstrated.

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EXPERIMENTS WITH EVIPAL IN PROLONGED ANESTHESIA*

A. H. Maloney, Ph.D., M.D., and R. Hertz, Ph.D., Washington, D. C.

THE need for a variety of nonvolatile anesthetics for surgery of short duration has been claimant. Evipal, the sodium salt of n-methyl cyclohexenylmethyl barbituric acid, is one of the newer products of synthetic chemistry designed to meet this need. It occurred to us that this hypnotic agent might also be rendered adaptable for use as an anesthetic for surgery of long duration. This report presents results which we have obtained on the use of evipal for prolonged anesthesia in rabbits and dogs.

Technic.—The animals were deprived of food for twenty-four hours preceding the experiment. The usual aseptic measures were employed whenever surgery was performed. An initial anesthetizing dose was given either intra-

*From the Department of Pharmacology, Howard University School of Medicine.
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peritoneally or by the slow intravenous method to the rabbit and intraperitoneally to the dog. The intraperitoneal method is similar in effect to the slow intravenous method but more uniform in action. In most instances the maintenance doses were given intravenously. A 5 per cent or 10 per cent aqueous solution of evipal was freshly prepared for each seance and administered at body temperature.

Two types of experiments were performed both with the objective of determining the possibilities of effective prolonged anesthesia, using fractional parts of the initial anesthetizing dose either at regular intervals or p.r.n.

In one type no surgery was done. Effective anesthesia was determined by response to pain from pinching the tip of the tail or pricking the animal at various sensitive points with a sharp needle. In the other type various surgical operations were performed. Two typical protocols for the rabbit and two for the dog are presented.

Exper. 1. Evipal 5 per cent solution
Male Rabbit, 2,300 grams

8:45 A.M. 100 mg./kg. initial dose, intravenously
8:50 A.M. Surgical anesthesia
9:05-10:35 A.M. 50 mg. q. ten minutes, intravenously
10:35 A.M. Respiration stopped; 5 mg. picrotoxin intravenously; prompt return of respiration
10:45 A.M. 50 mg., intravenously, respiration twenty-nine per minute
11:00 A.M. 100 mg., intravenously
11:15 A.M. 100 mg., intravenously
11:25 A.M. 50 mg., intravenously
11:35 A.M. 100 mg., intravenously
11:40 A.M. 100 mg., intravenously
11:50 A.M. 100 mg., intravenously
12:00 M. 300 mg., intraperitoneally. Surgical anesthesia continued until after 1:00 P.M. Following morning, living and well

In this experiment good surgical anesthesia was maintained during a period of more than four hours. No surgical measures were undertaken, but observations of the behavior of the animal were recorded. The arterial blood, as observed in the vessels of the ear, became slightly venous in color during the injection of the initial anesthetizing dose. Respiration which at the start was shallow became gradually deeper. In addition to response to pain, changes in rhythm and rate of respiration were cardinal signs either of escape from anesthesia or approach toward the danger zone. As time progressed the periods for symptomatic dosing grew more protracted. When the respiration came to a standstill, while evipal was being injected, picrotoxin was administered antidotally, and we observed a distinct clearing of the color of the arterial blood and improvement in the condition of the animal. In addition there was an absence of the signs of transitory respiratory embarrassment to subsequent evipal injections with no lessening of the degree of anesthesia. This observation suggested the rationality of the use of picrotoxin as a prophylactic measure either just before or following the initial anesthetizing dose of evipal. We tried it in a few cases with good results.

Exper. 7. Evipal 10 per cent solution
Female Rabbit, 3,700 grams
3:20 P.M. 110 mg./kg. intravenously, initial dose
3:25 P.M. Surgical anesthesia
3:25-3:40 P.M. Trachea and carotid artery cannulated and made ready for respiratory and pressure tracings
4:00 P.M. 200 mg. intravenously, total dose—record
4:15 P.M. 100 mg. intravenously, total dose—record
4:35 P.M. 100 mg. intravenously, total dose—record
4:45 P.M. 100 mg. intravenously, total dose—record
5:00 P.M. 100 mg. intravenously, total dose—record
hysterectomy performed
5:25 P.M. Sacrificed

This experiment demonstrated the successful performance of a prolonged surgical operation under evipal anesthesia. Throughout the exercise there was good anesthesia and good relaxation.

The tracing of the blood pressure record showed a slight inconsequential fall during each fractional injection. The experiment was of two hours' duration.

Exper. 2. Evipal 10 per cent solution
Male Dog, 11.5 kilos
11:45 A.M. 50 mg./kg. subcutaneously
12:50 P.M. Vomits, ataxic, staggers
1:30 P.M. 45 mg./kg. intraperitoneally
1:35 P.M. Narcosis, muscles relaxed
1:38 P.M. Anesthesia
2:00 P.M. 20 mg./kg., deep depression
2:40 P.M. 20 mg./kg., deep depression
3:25 P.M. 15 mg./kg., deep depression
4:30 P.M. Gross reflexes returning. Returned to cage; following morning apparently well; eating. Duration of surgical anesthesia two hours fifty minutes

This experiment demonstrated the slowness of the rate of absorption when evipal is administered subcutaneously, an observation which we have previously reported.[1] A 50 mg./kg. dose, which, given intraperitoneally would cause anesthesia in five to ten minutes, given subcutaneously produced simply stupor, incoordination and staggering. The duration of anesthesia was two hours and fifty minutes and recovery was complete.

Exper. 1. Evipal 10 per cent solution
Male Dog, 19.4 kilos
9:30 A.M. 50 mg./kg. intraperitoneally
9:35 A.M. Prostrate, muscle tone lost
9:42 A.M. Surgical anesthesia
9:50 A.M. 15 mg./kg. intraperitoneally, anesthesia maintained
9:57 A.M. 15 mg./kg. intraperitoneally, anesthesia maintained
10:00 A.M. Femoral vein dissected out, good anesthesia
10:05 A.M. Hypophysectomy by parapharyngeal approach
10:35 A.M. 10 mg./kg. intravenously
11:15 A.M. 10 mg./kg. intravenously

11:40 A.M. 10 mg./kg. intravenously
12:10 P.M. Died; hemorrhage into lungs

In this particular experiment a difficult operation was undertaken. An attempt was made to perform a hypophysectomy by the parapharyngeal approach. Unfortunately the animal died from hemorrhage, the anesthetic playing no part. The operation lasted two hours and twenty-eight minutes during which time anesthesia was smooth and uneventful. In Table I we have listed the dosages with effects on the individual rabbits and dogs used in this group of experiments.

TABLE I

NO.	WT. IN KG.	INITIAL DOSE OF EVIPAL MG./KG.	TOTAL DOSE IN MG.	TOTAL DOSE EVIPAL MG./KG.	DURATION OF ANESTHESIA IN MINUTES	REMARKS
			A—Rabbits			
1	2.3	100	1030	447	195	Picrotoxin restored arrested respiration; complete recovery
2	2.5	200	875	350	170	Complete recovery
3	2.37	21	375	158	70	Sacrificed following acute experiment
4	2.24	68	914	408	290	Picrotoxin given following initial dose of evipal. Complete recovery
5	2.25	47	490	192	127	Dosed symptomatically. Complete recovery
6	2.27	57	515	236	127	Picrotoxin restored arrested respiration; complete recovery
7	3.7	108	1000	270	120	Picrotoxin given preanesthetically. Trachea and carotid artery cannulated and hysterectomy performed, sacrificed
			B—Dogs			
1	19.4	51	2200	113.4	148	Acute experiment hypophysectomy
2	11.5	43	1580	137.4	217	Good anesthesia throughout, survived complete recovery
2(a)	11.5	43	900	78.2	172	Used three days after; survived, complete recovery
3	15	46	1650	110	62	Acute experiment tracheotomy and carotid artery, cannulated, sacrificed
4	13	46	1700	130.7	212	Given 6 mg. picrotoxin while in deep depression, good effect, complete recovery

DISCUSSION

These figures reveal the fact that the dog shows considerably less tolerance to evipal than the rabbit, the optimal anesthetic dose being 50 per cent less per kilo of body weight. The duration of action of the initial anesthetizing dose is longer and the maintenance doses are correspondingly smaller. This lessened tolerance finds expression in a graded reduction of optimal dosage requirements as one ascends the evolutionary scale in the development of the central nervous system, the recommended anesthetizing dose for man being about 10 mg./kg.

Regarding the effect of evipal on the blood pressure and respiration as revealed by kymographic records: during each injection the pressure in the carotid artery registers a fall below the preceding figure, the degree depending upon the rate of injection. Upon the cessation of the injection, however, it begins to make a gradual return but always stops just short (1 to 3 mg. Hg) of the antecedent level. Synchronously with the fall in blood pressure the respiration becomes slower and shallower, the volume tending to right itself without any significant increase in the rate. When death occurs, it is due to failure of respiration, since the heart continues to beat for a time with gradually diminishing effectiveness while the blood assumes a distinctly venous color. These eventualities could be avoided by use of the slow intravenous method and with the patient in the Fowler position.

In our first rabbit experiment we encountered a stoppage of respiration. Whether or not this condition would have corrected itself we do not know, but spontaneous respiration immediately followed picrotoxin injection. This experience was suggestive of the feasibility of picrotoxin prophylactically. It was tried both before and following the initial anesthetizing dose of evipal with uniformly good results. When so used, it prevents sudden paralysis of the respiratory center, resulting from too rapid intravenous injection. It also prevents the sudden drop in blood pressure from the same cause. We recommend that picrotoxin be routinely available whenever evipal is being employed for purposes of anesthesia. Respiratory depression and fall in blood pressure when not critical, but below the desired therapeutic level, may be overcome by an injection of metrazol, Jackson.[2] In several experiments, not here reported, we have substantially confirmed Jackson's observation using either coramine or metrazol. However, respiratory embarrassment is frequently peripheral, due to mechanical stoppage of the air passage. We frequently found it necessary to keep the tongue forward by continuous traction with a hemostat.

Evipal occasionally produces symptoms of excitement when given subcutaneously or even intraperitoneally in small doses. But when given intravenously the interval between introduction and narcotic action is too short for detectable signs of excitement. In this respect, viz., speed of action, evipal differs from pernocton, amytal, and nembutal. In man, due to a longer course of the systemic circulation, the time interval is longer, hence excitement might conceivably occur in rare instances. A few such instances have been recorded. Baetzner[3] attributes such induction excitement to underdosage. The Anesthetic Committee of the Medical Research Council[4] states that such conditions "have been seen only in unusually emotional subjects."

While evipal has established itself as a satisfactory agent for anesthesia of short duration, the above experiments have convinced us that it also has possibilities as an agency in the production of prolonged anesthesia. It was therefore most interesting to note during the preparation of this report that Baetzner[3] has tried fractional doses to prolong the period of anesthesia with no deleterious results.

SUMMARY

The results of experiments with evipal in the production of prolonged anesthesia on rabbits and dogs are presented.

1. Seven rabbits were kept under good surgical anesthesia with or without operative procedures for periods of 195, 170, 70, 290, 127, 120 minutes, respectively.

2. The total dose of evipal administered fractionally to each rabbit in mg./kg. was 447, 350, 158, 508, 192, 236, 270.

3. Four dogs (one used at two different times) were kept under good surgical anesthesia with or without operative procedures for periods of 148, 215, 172, 62, 212 minutes, respectively.

4. The total dose of evipal administered fractionally to each dog in mg./kg. was 113.4, 137.4, 78.2, 110, 130.7.

5. No untoward after-effects were observed in those animals that were not subjected to surgical procedures.

6. Picrotoxin used before or following the initial anesthetizing dose widens the margin of safety.

7. Gross observations and graphic records are discussed.

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STUDIES ON THE EFFECT OF THE ADMINISTRATION OF CAROTENE AND VITAMIN A IN PATIENTS WITH DIABETES MELLITUS*

I. The Effect of the Oral Administration of Carotene on the Blood Carotene and Cholesterol of Diabetic and Normal Individuals

Elaine P. Ralli, M.D., Harold Brandaleone, M.D., and Theodore Mandelbaum, M.D., New York, N. Y.

THE presence of carotene in the blood was established as early as 1913 by Van den Bergh and Snapper.[1] In 1919, Hess and Meyers[2] defined the state of an increase of carotene in the blood as carotinemia. Since then the condition has been reported clinically by several observers[3, 4, 5] and particular attention has been called to its presence in patients with diabetes.[6, 7, 8] Rabinowitch[9] has suggested that a high blood cholesterol is related to an increase in blood carotene. The importance of carotene in nutrition has been given prominence because of the fact that it is the outstanding precursor of vitamin A. That carotene will cure vitamin A deficiency has been definitely established by Moore[10] and others. Apparently in the body it is converted by vitamin A by the liver.[10] In view of these facts and because of the tendency for a high blood carotene in the diabetic patient, the relative effect of the oral administration of carotene in oil and of carrots on the blood carotene and cholesterol of normal and diabetic individuals was investigated.

PROCEDURE

The nondiabetic patients were given constant diets containing no eggs or carrots. At the end of several weeks, during which the blood carotene was determined at intervals, each subject received 20 c.c. of a 0.3 per cent solution of carotene† in oil. In view of the fact that carotene is best absorbed when given with a certain amount of fat, it was administered with 20 gm. of lard spread on two slices of white bread. At the same time the patient was given 200 c.c. of coffee with 30 gm. of glucose. This meal was adopted so that a known amount of carbohydrate could be given the diabetic patients. When carrots were used, 200 gm., cooked to the point of softness, were given with the same breakfast. Blood was then taken at intervals of 1, 3, 5, 10, 24, 30, 48, and 96 hours. After the five-hour specimen, the patient was fed the same type of diet which

*From the Third (New York University) Medical Division, Bellevue Hospital and the Diabetic Clinics of the University and Bellevue Hospital Medical College, New York University.

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†The carotene used was supplied by the S. M. A. Corporation and contained approximately 80 per cent beta carotene. Biologically it was found to be equivalent to the S. M. A. crystalline carotene.

he had received during the preliminary period. During the remainder of the observational period he was fed at the usual intervals. Further specimens of blood were taken one week and in some cases two weeks after the original dose of carotene. As it soon became apparent that the blood carotene did not change within the first three hours, the one-hour sample was omitted in subsequent experiments.

Blood serum was analyzed for carotene by the method of White and Gordon[11] in which carotene, extracted from the serum, is matched against solutions of dichromate, so standardized that a 0.2 per cent solution of dichromate represents 100 dichromate units. Blood cholesterols were done by the Bloor colorimetric method,[12] in Cases 1 to 9 inclusive on whole blood and in the remaining cases on blood plasma. Whole blood was used in the earlier curves to avoid withdrawing excessive amounts of blood.

For the study of the effect of carotene on the diabetics, patients were chosen of about the same age groups as the normals, with both moderate and severe forms of the disease. The fasting blood carotene was established on a known diet of carbohydrate, protein, and fat from which carrots and eggs were omitted. On the morning of the test, the patient received the same breakfast and the same amount of carotene in oil or carrots as the normals. In some cases insulin was given before and during the period of observation, in others it was omitted as indicated in the tables. The carotene, cholesterol, and sugar of the blood were determined at the same intervals of time. All the diabetic patients received carrots and carotene in oil, and when possible, both with and without insulin.

Blood sugars are not reported as they bore no relationship to the blood carotene.

RESULTS

Table I summarizes the subjects studied and gives the average fasting blood carotene and cholesterols before any carotene was administered. The state

TABLE I

SUMMARY OF DIABETIC AND NORMAL INDIVIDUALS STUDIED

	CASE	NUMBER	SEX	AGE	WEIGHT POUNDS	HEIGHT INCHES	AVERAGE FASTING		DURATION OF DIABETES	DIET IN GRAMS			INSULIN UNITS		
							CAROTENE MG. %	CHOLESTEROL MG. %		CARBOHYDRATE	PROTEIN	FAT	BREAKFAST	LUNCH	SUPPER
Diabetics	A. G.	7	M	47	127	63	.318	272.	7 years	250	65	85	15	0	15
	B. R.	8	M	45	120	68	.216	204.	3 months	200	75	85	20	0	15
	A. C.	9	M	48	140	70	.337	211.	9 months	250	75	85	30	10	25
	M. E.	10	M	40	190	66	.207	241.	3 years	180	65	85	10	0	5
	P. S.	11	M	65	104	60	.337	251.	7 years	180	75	85	0	0	0
Normals	H. F.	1	M	50	151	68	.122	158.							
	P. F.	2	M	45	141	62	.077	176.							
	J. C.	3	M	48	122	65	.122	190.							
	J. P.	4	M	44	148	69	.108	163.							
	S. K.	5	M	22	132	64	.136	172.							
	W. McD.	6	M	34	158	70	.054	165.							

TABLE II

EFFECT OF 20 C.C. OF .3 PER CENT SOLUTION OF CAROTENE IN OIL ON BLOOD CAROTENE AND CHOLESTEROL OF NORMAL INDIVIDUALS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK	NOTES
1	Carotene	.108	.135	.122	.135	.135	.122	.149	.162	.149	.149	
	Cholesterol	168.	161.	180.	192.	183.	173.	161.	156.	175.	147.	
2	Carotene	.068	.108	.108	.095	.108	.108	.122	.162	.135	.108	
	Cholesterol	179.	200.	192.	200.	200.	189.	156.	156.	151.	164.	
3	Carotene	.135	.149	.149	.149	.176	.216	.203	.203	.203	.216	
	Cholesterol	247.	238.	238.	192.	208.	197.	206.	216.	200.	172.	
3	Carotene	.216	.203	.203	.203	.216	.216	.216	.203	.176	.189	7 days after administration of carotene for Curve 1
	Cholesterol	172.	208.	211.	211.	193.	200.	200.	238.	229.	187.	
4	Carotene	.135	.122	.122	.122	.176	.216	.216		.203	.203	
	Cholesterol	229.	211.	215.	204.	231.	225.	178.	197.	166.	153.	
4	Carotene	.203	.203	.216	.203	.243	.243	.243	.230	.216	.216	7 days after administration of carotene for Curve 1
	Cholesterol	153.	196.	215.	199.	222.	220.	199.	234.	219.	200.	
5	Carotene	.122	.122	.122	.149	.189	.162	.176	.203	.162	.108	
	Cholesterol	166.	152.	188.	158.	178.	174.	179.	162.	165.	158.	
5	Carotene	.095	.095	.095	.108	.108	.122	.108	.122	.135	.135	13 days after administration of carotene for Curve 1
	Cholesterol	159.	165.	160.	151.	141.	154.	158.	152.	163.	159.	
5	Carotene	.149	.149	.149	.149	.203	.149	.149	.149	.162	.149	14 days after administration of carotene for Curve 2
	Cholesterol	154.	136.	161.	180.	194.	166.	152.	197.	137.	160.	
5	Carotene	.149	.149	.149	.189	.203	.203	.189	.176	.176	.122	This curve was made after the subject received 1 c.c. of carotene daily for 1 month
	Cholesterol	152.	161.	160.	131.	179.	149.	163.	174.	156.	146.	
6	Carotene	.068	.068	.081	.095	.095	.122	.122	.122	.095	.068	
	Cholesterol	172.	143.	172.	139.	185.	163.	172.	156.	162.	161.	
6	Carotene	.054	.054	.068	.068	.068	.081	.081	.081	.081	.081	14 days after administration of carotene for Curve 1
	Cholesterol	150.	156.	150.	143.	143.	151.	166.	137.	148.	163.	

of nutrition may be estimated from the heights and weights of the patients. In a study involving an essential food, such as vitamin A, it is important that the subject be in good health so that the vitamin or its precursor is not required to satisfy some nutritional deficiency. Tables II and III show the effects, on the blood carotene and cholesterol of normal individuals, of a .3 per cent solution of carotene in oil and 200 gm. of carrots. Tables IV and V give the results on the diabetic patients.

The fasting blood carotene was consistently higher in diabetics than in normals. The one exception to this was Case 14, whose average fasting carotene was .133 mg. per cent. In an earlier series of observations[13] the average fasting blood carotene in normals was found to be .109 mg. per cent, in diabetics .262 mg. per cent. Following the administration of carotene in oil to the nondiabetics there was a rise in the blood carotene which tended to reach a peak after twenty-four hours. In two cases this occurred earlier, in four cases it was delayed to thirty hours or more. At the end of ninety-six hours, the fasting carotene was at or only slightly above the original level in nine of the twelve experiments.

TABLE III

EFFECT OF 200 GM. OF CARROTS ON BLOOD CAROTENE AND CHOLESTEROL OF NORMAL INDIVIDUALS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK
1	Carotene	.108	.122	.135	.122	.108	.149	.135	.122	.108	.108
	Cholesterol	150.	168.	188.	177.	161.	185.	185.	173.	217.	179.
2	Carotene	.108	.108	.108	.108	.095	.108	.108	.095	.081	.081
	Cholesterol	145.	186.	203.	182.	167.	200.	189.	179.	208.	164.
3	Carotene	.162	.189	.189	.189	.189	.203	.203	.162	.149	.135
	Cholesterol	211.	203.	227.	213.	201.	202.	214.	205.	194.	185.
5	Carotene	.095	.108	.095	.095	.081	.095	.095	.108	.095	.122
	Cholesterol	177.	153.	161.	151.	147.	165.	147.	152.	144.	166.
6	Carotene	.054	.054	.054	.054	.054	.054	.054	.068	.054	.068
	Cholesterol	177.	147.	158.	143.	151.	150.	168.	160.	156.	172.

The curves for the normals after both carotene and carrots show a certain constancy. In the diabetics the responses were much less regular. Starting at a higher level there was in most of the cases a more precipitous rise, which became evident within the first ten hours in six experiments. This was followed by a definite drop, and in three experiments by an even greater rise at twenty-four or thirty hours. The absolute rise was greater in the diabetic patients after carotene and carrots. This bore no relation to the severity of the disease, but the patients who had the disease longest tended to have the greatest rise in blood carotene after receiving carotene in oil.

Carotene in oil or carrots was administered more than once to certain subjects in the diabetic and nondiabetic groups. In the normals (see Tables II and III), the second administration of carotene was not usually followed by as great a rise in the blood carotene level as it was in the diabetics. In the diabetics (Tables IV and V) the second curve, with the exception of Case 8, was on a higher level than the first and tended to remain elevated at the ninety-sixth hour. Case 7, diabetic, received carotene in oil three times at intervals of two weeks and the blood carotene was further elevated after each administration. A

TABLE IV

EFFECT OF 20 C.C. OF .3 PER CENT SOLUTION OF CAROTENE IN OIL ON BLOOD CAROTENE AND CHOLESTEROL OF DIABETIC PATIENTS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK	ADMINISTRATION OF INSULIN DURING CURVES
7	Carotene Cholesterol	.284 274.	.338 252.	.459 238.	.459 255.	.459 253.	.324 207.	.351 217.	.351 247.	.311 247.	.405 227.	No insulin for one week, 20 units after 7½ hours
7	Carotene Cholesterol	.567 287.	.594 283.	.459 317.	.513 305.	.459 287.	.567 342.	.567 325.	.513 346.	.594 291.	.513 272.	15 units before fasting, 10 units before 10 hr. specimens
7	Carotene Cholesterol	.378 278.	.513 250.	.540 253.	.540 218.	.567 294.	.513 256.	.513 250.	.540 266.	.675 329.	.486 313.	No insulin during curve
8	Carotene Cholesterol	.284 214.		.297 184.	.284 236.	.297 260.	.270 205.	.257 197.	.324 231.	.324 272.	.324 216.	15 units before 10 hr. specimen
9	Carotene Cholesterol	.284 179.		.311 225.	.392 223.	.351 184.	.297 221.	.270 219.	.351 208.	.311 212.	.297 175.	20 units before 5 hr., 30 units before 10 hr. specimens and 10 units at midnight
10	Carotene Cholesterol	.203 200.		.230 203.	.230 219.	.230 212.	.230 195.	.230 250.	.230	.243	.230 230.	8 units before 10 hr. specimen
11	Carotene Cholesterol	.486 195.		.562 256.	.562 250.	.594	.513 223.	.540 301.		.594 258.	.648 291.	No insulin
12	Carotene Cholesterol	.297 161.		.297 168.	.324 227.	.338 223.	.311 176.	.311 210.		324 205.	.311 208.	8 units before fasting specimen

TABLE V

EFFECT OF 200 GRAMS OF CARROTS ON BLOOD CAROTENE AND CHOLESTEROL OF DIABETIC PATIENTS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK	ADMINISTRATION OF INSULIN DURING CURVES
7	Carotene	.486	.486	.459	.513	.486	.486	.486	.459	.567	.513	Insulin 15 units before fasting specimen. 10 units before 10 hour specimen
	Cholesterol	281.	313.	305.	321.	253.	287.	329.	256.	283.	269.	
7	Carotene	.513	.513	.567	.567	.567	.540	.513	.486	.513	.567	No insulin during observation. Last dose 24 hours before curve
	Cholesterol	269.	321.	352.	329.	294.	305.	313.	263.	325.	287.	
8	Carotene	.216	.189	.216	.243	.257			.284	.297	.270	No insulin for first 24 hours of curve
	Cholesterol	203.	176.	188.	188.	192.	190.	174.	195.	258.	260.	
8	Carotene	.270	.257	.257	.270	.284	.297	.284	.284	.284	.284	Insulin 20 units before fasting. 10 units before 10 hour specimen
	Cholesterol	260.	243.	216.	247.	266.	284.	281.	250.	278.	214.	
9	Carotene	.284	.297	.284	.311	.324	.297	.297	.311	.297	.284	30 units before fasting, 10 units before 5 hour and 25 units before 10 hour specimens
	Cholesterol	214.	210.	200.	233.	236.	210.	192.	198.	210.	179.	
10	Carotene	.203		.203	.149	.189	.230	.216	.203	.162	.203	15 units before fasting, 10 units before 10 hour specimen
	Cholesterol	219.		216.	219.	217.	233.	203.	212.	217.	200.	
11	Carotene	.351		.392	.392	.392	.351	.365	.392	.392		No insulin
	Cholesterol	223.		229.	205.	207.	194.	227.	165.	184.	181.	
12	Carotene	.284		.338	.338	.324	.297	297	.297	.284	.297	8 units before fasting specimen
	Cholesterol	159.		158.	148.	153.	151.	197.	159.	166.	165.	

similar series of experiments was done on Case 5, a nondiabetic patient. He received three doses of carotene and one of carrots. The second curve was at a definitely lower level than the first. The third started at a higher fasting level, showed a drop and did not rise above the fasting level. The patient was then placed on 1 c.c. of a 0.3 per cent solution of carotene for thirty days after which another dose of 20 c.c. of carotene was administered. In the fourth experiment, the fasting blood carotene was .149 mg. per cent, which was only .027 mg. per cent higher than the original fasting level. This, however, reached a peak of .203 mg. per cent at twenty-four hours and was maintained until the forty-eighth hour, when it began to fall gradually. The fall was slower than in the previous experiments. This curve simulated the type of curve observed in

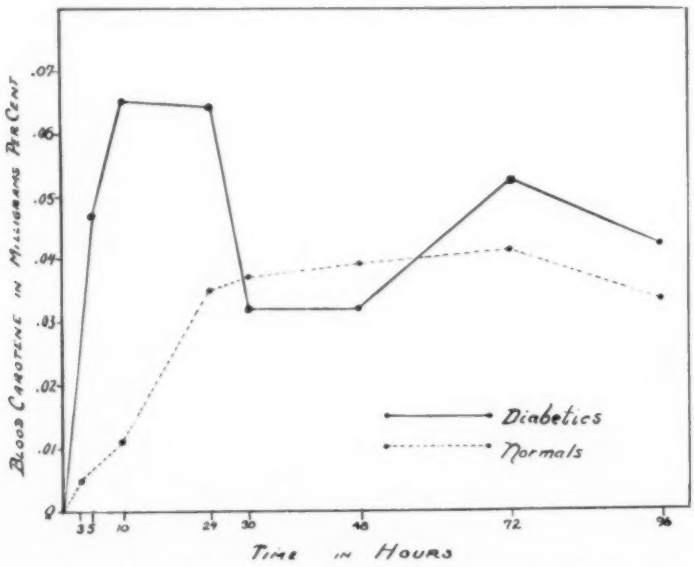

Chart 1.—Curves representing absolute rise in milligram per cent of blood carotene above fasting levels, in normal and diabetic individuals following the administration of carotene. These figures represent the mean values obtained from the figures in Tables II and IV.

the diabetic patients, but to obtain this effect the patient received, in the preceding period in experiments and daily doses, a total of 90 c.c. of carotene in oil.

Concerning the relation of the blood carotene to the blood cholesterol, it was found that prior to any administration of carotene or carrots (Table I) the blood cholesterol in the diabetics was higher than in the normals. There was also a tendency for the high blood carotenes to be associated with higher blood cholesterols. Following the administration of carotene in oil to normals, the blood cholesterol rose slightly and had returned to the fasting level or very nearly so by the end of the curve. If the figures are averaged, there is a tendency for the cholesterol to follow the carotene level, but analyzed individually this is not so. In the diabetics, although the cholesterol level was higher, it did not, following the administration of carotene or carrots, bear any direct relationship to the carotene level and the highest blood carotenes were not

accompanied by the highest blood cholesterols. In this connection it is well to realize that the curves covered a period of ninety-six hours during which time other meals were eaten.

DISCUSSION

The higher fasting blood carotene in the diabetics and their reaction to the ingestion of carotene may be explained either on the concentration of carotene in the liver at the time carotene is administered or on a concentration of other highly unsaturated substances. In support of the suggestion that the concentration of carotene may be increased in the livers of diabetics the analyses of the livers of 8 diabetic patients and 13 nondiabetics, for vitamin A and carotene is reported (Table VI). The extraction of the liver oil was done by the method described by Moore[10] and the readings, made in a Lovibond tintometer, are reported in yellow units. The antimony trichloride reaction[14] was used for the determination of vitamin A and the results are reported in blue units. Other observers have reported the vitamin A content of human livers, but unfortunately not the carotene content.[15, 16] Of the 8 diabetic patients all but two had more than 1,200 yellow units per 100 gm. of liver. Of these 2, one had hemochromatosis with pigment deposit in the liver, which might have interfered with the absorption of carotene by the liver. Only 4 of the 13 nondiabetic patients had an increased concentration of carotene in their livers, the other 9 having 600 yellow units or less per 100 gm. of liver. These figures, although not conclusive, show a tendency for an increased concentration of carotene in the livers of diabetic patients.

TABLE VI

VITAMIN A AND CAROTENE CONTENT OF HUMAN LIVERS REPORTED IN BLUE UNITS (VITAMIN A) AND YELLOW UNITS (CAROTENE)

CONDITION OF PATIENT.	BLUE UNITS PER 100 GM.	YELLOW UNITS PER 100 GM.
Diabetic patient	9,680	9,680
Diabetic patient	14,520	520
Diabetic and Hemochromatosis	1,905	120
Diabetic patient	22,880	1,200
Diabetic patient	84,480	1,920
Diabetic patient	48,400	1,200
Diabetic patient	61,380	1,400
Diabetic patient	48,400	5,000
Nondiabetic patients		
Pulmonary edema	16,500	600
Stabbed	8,800	400
Auto accident	35,200	560
Found dead	112,640	480
Pulmonary thrombosis	17,600	640
Apoplexy	56,320	2,560
Sarcoma, metastases to liver (child)	10,560	1,210
Congestive heart failure	55,000	3,750
Stabbed	19,050	500
Lobular pneumonia	9,900	500
Congestive heart failure	19,800	350
Sickle-cell anemia	12,100	150
Accident	24,200	1,400

In considering the reasons for increased concentration of carotene in the livers of diabetic patients, two possibilities suggest themselves: the first is a matter of ingestion; the diet of the diabetic might be higher in vegetables and so provide more carotene per se or substances which could be built up into carotene; second, the conversion of carotene to vitamin A may be interfered with in the liver of the diabetic patient.

In this group of diabetic patients four (Cases 7, 10, 11, 12) were clinic patients who had been under observation for periods of from three months to three years. During this time, they averaged 400 to 500 gm. of vegetables daily. The other two patients had, as far as could be established, developed their diabetes in the preceding twelve months, during which time they had eaten an average normal diet. Therefore in four of these cases it is possible to state definitely that the vegetable intake for even long periods of time was not really excessive. It is true that a rise in blood carotene will result from the excessive ingestion of the pigment,[17] but under ordinary circumstances the normal individual can manage to control this, as is shown by the effect of carrots and carotene on the normal group. Furthermore, as shown in Case 5, it was only after receiving a total of 90 c.c. of carotene in oil that it was possible to maintain an elevated blood carotene. Thus the excessive ingestion of vegetables, although a factor in producing an increase in blood carotene, does not seem to offer the most likely explanation.

The second suggestion, that carotene accumulates in the liver of the diabetic because of some interference with its conversion to vitamin A, would mean that a rise in blood carotene could result from the accumulation of carotene in the liver and the consequent inability of this organ to absorb as much from the blood. Moore[10] and others have shown that the conversion of carotene to vitamin A occurs in the liver, and the evidence is reasonably strong that the change is essentially a hydration of the central double bond of the carotene molecule. In 1933 we reported[18] that carotene fed daily to four depancreatized dogs, three received it as carotene in oil and one in two egg yolks, was not converted to vitamin A, as it was in a control nondiabetic dog, previously kept on an A deficient diet. The blue to yellow ratio of the control dog was 587:1. In the four depancreatized dogs it was 11:1, 176:1, 121:1, and 8:1. In two dogs kept on a mixed diet of kitchen scraps it was 1,000:1 and 880:1. Thus the normal dogs, receiving a mixed diet, and the control dog, devitaminized and then fed carotene, had a greater proportion of vitamin A in their livers than did the depancreatized dogs fed carotene.

At the present time we are inclined to feel that the accumulation of carotene in the liver of the diabetic is due to a diminished ability on the part of the liver to convert carotene to vitamin A. This does not mean that hydration of the carotene molecule does not occur at all, but that it may be slowed up sufficiently to allow carotene to accumulate in the liver. When this occurs, a rise in the level of carotene in the blood follows.

SUMMARY AND CONCLUSIONS

The fasting blood carotene was found to be higher in 8 of 9 patients with diabetes mellitus than in a group of 9 nondiabetic individuals.

Following the administration of carotene in oil and of carrots, the blood carotene rose sooner in the diabetic patients, the increase was greater and was maintained for a longer period of time.

A second administration of carotene to nondiabetic patients did not result in any greater increase in the blood carotene than did the first dose. In the diabetic patients a second dose of carotene caused a greater increase in blood carotene in all but one case.

To obtain a curve in a normal simulating that in the diabetic patients, it was necessary to administer large amounts of carotene, Case 5 demonstrating this. The prolonged administration of 1 c.c. of carotene in oil to three normal and three diabetic patients resulted in a greater increase in the blood carotene in the diabetic patients and a more gradual fall.

The fasting blood cholesterol was higher in the diabetic patients but bore no absolute relationship to the height of the blood carotene. The blood sugar bore no relationship to the blood carotene.

To explain these results the hypothesis is advanced that there is an increased concentration of carotene in the liver of the diabetic patient due to a diminished ability on the part of this organ to convert carotene to vitamin A, and that this increased concentration interferes with the absorption of carotene from the blood.

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LABORATORY METHODS

THE TECHNIC AND APPLICATION OF SUPRAVITAL STAINING*

EDWARD A. GALL, M.D., BOSTON, MASS.

THE staining of blood preparations for the microscopic study of the living cell is a procedure which has come rapidly to the fore in recent years. It is by no means a new discovery, of course, but its practical application was not fully appreciated until Simpson (1919-1921),[42, 43] Sabin (1921),[34] and Sabin, Doan and Cunningham (1922-1925)[9, 35, 36, 37] utilized the method for the determination of individual cell characteristics and their embryologic development. Since this time a host of workers have demonstrated both the value and limitations of this technic.[21, 45]

Prior to Ehrlich's introduction of the tri-acid stain, the study of the unstained blood in the living state was the only method generally available. Bettmann,[3] Rosin and Bibergeil,[32, 33] and Israel and Pappenheim[22] first used neutral red, a relatively nontoxic organic dye, in their studies of red blood cells. Following this, very little work of moment was done with nontoxic dyes other than the vital staining of cells in situ in the living animal by Aschoff and Kiyono.[1] Cowdry, in 1914, first demonstrated the specificity of Janus green for the staining of cellular chondriosomes (mitochondria).[10]

Subsequent to the publications of Sabin, Doan and Cunningham, there has been a progressive increase in the utilization of the warm box and the dyes, neutral red and Janus green, for the purpose of what has been termed supravital staining in the investigation of cellular morphology in disease and health. Technically, this refers to the staining of cells after somatic death (following removal from the body) but before cellular (intrinsic) dissolution. For the most part, the fundamental literature generally available concerning this subject is the production of former students and colleagues of these initial investigators in the field. As such, they have tended to refer for more adequate and detailed description of specific morphology to the original presentations of their predecessors. In those papers in which well-defined descriptive effort is made, too often there is the tendency to leave unsaid that which presumably may be found in the original publications. This naturally makes for considerable difficulty to the novice in this phase of blood study. Furthermore, many of these introductory works are not readily procurable, and many of those which are, vary greatly in the interpretations of the fundamental elements described.

*From the Medical Clinic of The Boston Dispensary (Service of Dr. Joseph H. Pratt.), and Department of Medicine, Tufts Medical School.

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For this reason this compilation of the descriptions of the commonly observed human blood cells stained supravitally in both normal and pathologic states is presented. Personal observation has been utilized as the encompassing influence. Such cells as have not been personally observed are demonstrated by means of analyses of the literature.

Method.—For the sake of completeness a brief résumé of the method of staining as outlined by Forkner is appended.[19] For ordinary peripheral blood study the following solutions are necessary:

Solution I: 0.125 gm. of vital neutral red[7] ("Toluylene Red," National Aniline and Chemical Co.) is dissolved in 50 c.c. of neutral absolute ethyl alcohol, thus producing a 0.25 per cent solution which is saturated.

Solution II: 0.125 gm. of vital Janus green[7] ("Diazine Green," National Aniline and Chemical Co.) is dissolved in 62.5 c.c. of neutral absolute ethyl alcohol. This is a 0.20 per cent solution which likewise is saturated.

Solutions I and II, if well stoppered, may be kept for indefinite periods as stock solutions. They must, however, be kept dust-free by filtration from time to time. Since Janus green and neutral red in combined solution rapidly deteriorate, it is advisable not to combine the two until just prior to application.[19, 36]

Solution III: 50 drops of Solution I are added to 10 c.c. of neutral absolute ethyl alcohol. This preparation may be utilized for the staining of blood with neutral red alone.

Solution IV: 2 drops of Solution II are added to 3 c.c. of Solution III. This is the solution which is commonly used, since it will identify by stain specificity many more of the characteristic cellular features than Solution III alone. This combination has been used throughout this study, save where more detailed observation of uninhibited motility was to be made.

It has been the author's custom to use ordinary glass slides (75 by 25 mm.). Larger and thinner cover slips (22 by 40 mm.) than those more frequently used have been found to be advantageous in that they produce a thinner and more even spread. The glassware must be scrupulously clean, for the most minute contamination will serve to produce unsatisfactory preparations. Cleansing is accomplished in ordinary cleaning solution (potassium dichromate and sulphuric acid) for twenty-four to forty-eight hours. The glassware is then washed in running tap water for one to two hours, rinsed first in distilled water, next in 70 per cent alcohol, and finally stored in 50 to 70 per cent alcohol until ready for use. At this time slides and cover slips are dipped in clean ether, allowed to dry in air, and then polished vigorously with clean silk. It has been suggested by Sabin that the use of jeweler's rouge will give a higher polish.[36] This has not been found to be appreciably advantageous for routine use.

The slide is swept lightly with a soft camel's hair brush to remove such silk or dust particles as may adhere. It is then lightly flamed and its surface is flooded with Solution III or IV. The dye is next permitted to drain back into its container, the excess stain at the edges is absorbed with blotting

paper, and the alcohol remaining on the surface will evaporate spontaneously. A very thin, smooth film of violaceous pink stain should remain on the slide. A blotchy smear signifies the presence of grease and such a slide must be discarded. Stained slides may be stored for future use in a dust-free container. The precaution should be taken, however, to mark one end of the unstained surface so that identification will be more readily made.[45]

Blood is drawn from the finger or ear by ordinary needle puncture. The skin is prepared for puncture by washing with soap and water, after which the soap is entirely removed with running water. It is then wiped with alcohol and finally with ether. A dry surface is essential. The cover slip is held between the examiner's finger tips, care being taken to allow no part of the flat surface to be touched by skin. The surface is swept lightly with a soft brush and applied cautiously to the freshly risen blood drop. The cover slip is then promptly permitted to drop gently upon the surface of the stained smear. No additional pressure is to be exerted to cause the blood film to spread. If it does not do so spontaneously, the preparation should be discarded. Directly the film has spread, the edges of the cover slip are rimmed with either salvoline or ordinary white vaseline. Here again care must be taken not to press upon the cover slip or cause it to revolve upon the surface of the slide. The preparation is now ready for use.

The slide is placed upon the stage of the microscope which has been previously warmed to body temperature (36 to 38° C.) in a warm box. Fifteen or twenty minutes usually suffice to permit the cells to recover from the trauma of transportation and temperature change, and also to absorb a sufficient amount of dye to allow identification.

A simple warm box may be handily manufactured at a nominal cost. Its four sides are made approximately 24 inches long, 15 inches wide, and 11 inches in height. It is covered above by two lateral boards to which are hinged two central covers which are cut to fit about the tube of the inserted microscope. On the rear wall of the box there are arranged three carbon bulbs which are controlled by a four-way switch so that any number may be used to regulate the temperature within. The walls of the box are insulated by a perforated asbestos lining. The fore side of the box has two lateral armholes which are closed by asbestos or rubber curtains and a central peephole to allow for proper approximation of the microscope objective to the slide. At one side there is placed a small hole for the passage of wire for illumination and another for the insertion of a thermometer. The entire apparatus and its production should cost no more than two or three dollars.

Such elementary data are prefixed not superfluously, since much of the critical literature directed at this procedure is resultant upon minor errors in technic.

Care must be taken to avoid pressure of the objective upon the cover slip. It is good policy to examine the smear with the low power lens to determine whether or not there is an adequate spread in the field to be examined. The objective is then lowered into the oil under direct observation through the peephole, raised slightly, and then focused gently.

The best criteria one may adopt for the determination of preparation excellence are the presence of a fairly regular distribution without air bubbles, the regular contours of the red blood cells, the absence of nuclear staining, and the continued motility of the active leucocytes. Mitochondria are stained by Janus green, a brilliant blue green. Neutral red varies its staining characteristics with the pH of the structures stained, from light yellow to deep maroon.

Morphology.—Red Blood Cells: Erythrocytes appear morphologically as in the unstained state, occasionally greenish in color, but more often exhibiting a straw yellow tint. There is no inherent motility. Fluid currents, however, will carry the cells smoothly across the field, changing their shapes as they adhere transiently to static objects. Thereafter the cells rapidly resume their rounded form. Older preparations exhibit clumping, some disintegration, and general crenation. The normal cell border is distinct, smooth, and rounded but the central pallor is less often observed than in unstained preparations. Neutral red and Janus green will both stain reticular substance in less mature cells, but only in a concentration which would be too toxic for routine use.[36] The same applies to parasitic inclusion bodies. In most bloods, however, small globular, refractile, homogeneous bodies staining bright red may be observed to overlie or lie within the stroma of an occasional red cell. There is usually a single body in such a cell, but as many as 3 to 6 have been observed. These bodies frequently dance actively within the bounds of the cell border and are rarely seen beyond it. It is possible that they are extruded bits of oxyphilic hemoglobin or merely artifacts. They do, however, appear in approximately 0.1 to 1.0 per cent of red blood cells in normal bloods, but have been observed as high as 10 to 15 per cent in spreads from patients who have evident bone marrow stress (myelogenous leucemia, chronic hemorrhagic anemia, and metastatic bone cancer). Similar globules have been described previously by Isaacs.[49] Not infrequently there are present in the same erythrocyte, apparently joined to the globule, small, fine, granular shreds stained greenish blue. Less often this substance occurs alone. There is no evident relationship of the latter to the specific material within the reticulocyte.

The *Megaloblast* has never been observed in peripheral blood by the author. It is said to be a nonmotile cell with a hazy, tenacious border, considerably larger than the mature erythrocyte. The nucleus, usually round, may be oval or indented and is centrally placed. It has a slim, fine border and a sparse meshwork of chromatin in which may be observed a large vacuolar nucleolus and rarely two. The nucleus is quite large in proportion to the size of the cell and often covers as much as two-thirds of its flat surface. Mitosis has been recorded. Characteristic is the earliest faint glow of hemoglobin in the cytoplasm. The latter is nongranular and homogeneous save at the border of the nucleus where some mottling may appear. No structures stained with neutral red are described. There are, however, large numbers of coarse, rod-shaped and coccoid mitochondria scattered without pattern throughout. Characteristically, at this state the Janus green stain does not persist and after some fifteen minutes the color is lost, and the mitochondria

TABLE I

	ERYTHROBLAST	NORMOBLAST	MONOBLAST	MYELOBLAST	MYELOCYTE	MONOCYTE	LYMPHOCYTE	LYMPHOBLAST	POLYMORPHONUCLEAR NEUTROPHILE	CLASMATOCYTE	EOSINOPHILE	BASOPHILE
Size	8 – 12 μ	7.5 – 10μ	9 – 18 μ	10 – 12 μ	8 – 12 μ	10 – 15 μ	6 – 14 μ	8 – 10 μ	8 – 12 μ	10 – 80 μ	8 – 12 μ	7 – 10 μ
Motility	Nonmotile	Nonmotile	Nonmotile	Nonmotile	Nonmotile	Nonmotile with J. G. Motile with N. R. alone. Slow; from one position in 10-15 minutes. Surface film rolling	Rarely motile with J. G. Intermediate form usually. Slow rate. Flows through constrict. bands with nucleus ahead 4-15 μ/min. at 37° C.	Round Nonmotile	Actively ameboid. Irreg. course 34 μ per minute at 37° C. Persists	Motile as Mono	Motile. Ameboid Sl. slower rate than poly but immobile in 30-45 minutes	Motile Rate as high or higher than poly Persists longer than eos. but not as long as poly
Border	Rounded Distinct	Round or oval Distinct Bulge of extruding nucleus	Fine and smooth Irregular Bleblike excres. rather than filiform processes (Latter occasional)	Rounded Thin and tenacious No processes	Round or oval Thin Distinct No pseudopodia	Irregular Faint spikelike processes waving, and bleblike pseudopodia	Round or oval Distinct No pseudopodia Constriction only when motile	Distinct No pseudopodia	Irregular Repeated pseudopod formation May stretch out to great length	Smooth Inconstant filiform and bleblike protrusions	As in poly.	As in poly
Cytoplasm	Homogeneous Yellow tint (Hb). Gel	Homogeneous Full complement of hemoglobin Gel	Slightly foamy Ground-glass Gel	Hazy Slightly mottled Gel. No movement	Homogeneous gel. Sol in late Myelocyte "C"	Foamy, ground-glass Clear surface film Included bodies move about No streaming Sol	Clear and glassy Homogeneous Yellow tint in younger forms Sol	Clear Homogeneous Faint yellow tint Gel	Homogeneous and clear Ecto-and endoplasm streaming Granules rare in ectoplasm Sol	Abundant Clear Sol	Clear and homogeneous Well-defined ectoplasm Sol	Clear and homogeneous No ecto-or endoplasm Sol
Nucleus	Eccentric Small ⇔ cytoplasm Chromatin dense Single nucleolus Occas. mitosis	Eccentric or extruding Round Chromatin very dense and structureless	Oval or indented (Occas. other shape) Chromatin poorly developed, hardly visible 1–2 nucleoli occas.	Round (occas. indented) Eccentric Membrane thin, imperceptible. Chromatin loose meshwork Nucleoli 2-4	Round or indented As blast Membrane more distinct	Eccentric Round-horse-shoe-bizarre Membrane distinct Chromatin loose regular mesh Soapsuds appearance	*Large Lymph:* Eccentric Thick mesh concent. at membrane Chromatin coarse Round, oval or indented *Small Lymph:* Central Takes up most of cell	Small and round Eccentric Membrane fine, distinct Chromatin loose mesh, granular 1-2 nucleoli	Polynuclear. Shape compressed Drags behind with cell flow	Round or oval (never horse-shoe) Central Chromatin faint, scattered clumps May be 2-3 nuclei	Usually bilobate Dragged as in poly	Rounded or lobed Lobes folded—do not stretch. At fore end of cell with motion

TABLE I—Continued

	ERYTHROBLAST	NORMOBLAST	MONOBLAST	MYELOBLAST	MYELOCYTE	MONOCYTE	LYMPHOCYTE	LYMPHOBLAST	POLYMORPHONUCLEAR NEUTROPHILE	CLASMATOCYTE	EOSINOPHILE	BASOPHILE
Neutral Red	Occasional Superimposed red globules (Refractile)	As in erythroblast	None Premonocyte has few brick red clumped near centrosphere (Fixed here)	None	A = 3-10 scattered B = 50-100 C = Full complement Motion only in late "C" Refractile, bright red	40-80, vary from dust size to vacuole Nonrefractile Fresh brick red Diffusely scattered; occas. rosette All same color	Small: Has few (3-5), small size, scattered Large: Has relatively more—large gran. May clump Bright red—refractile	None	Stream and dance Fill endoplasm Tiny, round yellowish pink—refractile Occas. nonrefractile vacuole	Large numbers Move actively with no pattern Vary from dust particles to R. B. C. size Color varies from scarlet to maroon Nonrefractile	Stream and dance Oval, all same size Larger than poly Bright yellow Refractile	Stream and dance Fewer and smaller than eos Round, vary in size Deep maroon Nonrefractile
Janus Green	Small coccoid Gathered loosely at nucleus, often to one side	Fewer and smaller than erythroblast Sharply limited to one side of nucleus	Tiny, coccoid Diffusely scattered Occas. small clumps or large clump in nuclear hof	Slender threads and commas Constant size in same cell Diffusely scattered	A = Fine, coccoid, fill cell B = Fewer, mixed with N. R. C = Few at cell periphery only	Myriads; fine, dust-like coccoid Scattered diffusely	Many (10-60). Coarse, coccoid or rod Tend to clump at one side of nucleus with few scattered elsewhere	Short, plump rods Occas. scattered, more often closely adherent to nucleus Numerous	Few and fine Obscured by N. R.	Rare, if any	Rare	Rare, occas. small clump of tiny coccoid
Unstained Bodies	None	None	None	None	None	Occasional (rare) refractile mahogany granule	Refractile, mahogany granules (1-3) in 34%	Occas. (rare) refractile, mahogany granule	None significant	Unstained vacuoles, debris, etc.	None	None
Phagocytosis	----------	----------	----------	----------	----------	Phagocytic for particles Lie anywhere in cytoplasm peripheral to rosette	----------	----------	Phagocytic for debris, bacteris, etc.	Phagocytic for particles or whole cells. Injected particles come to lie at first, close to nucleus	----------	----------

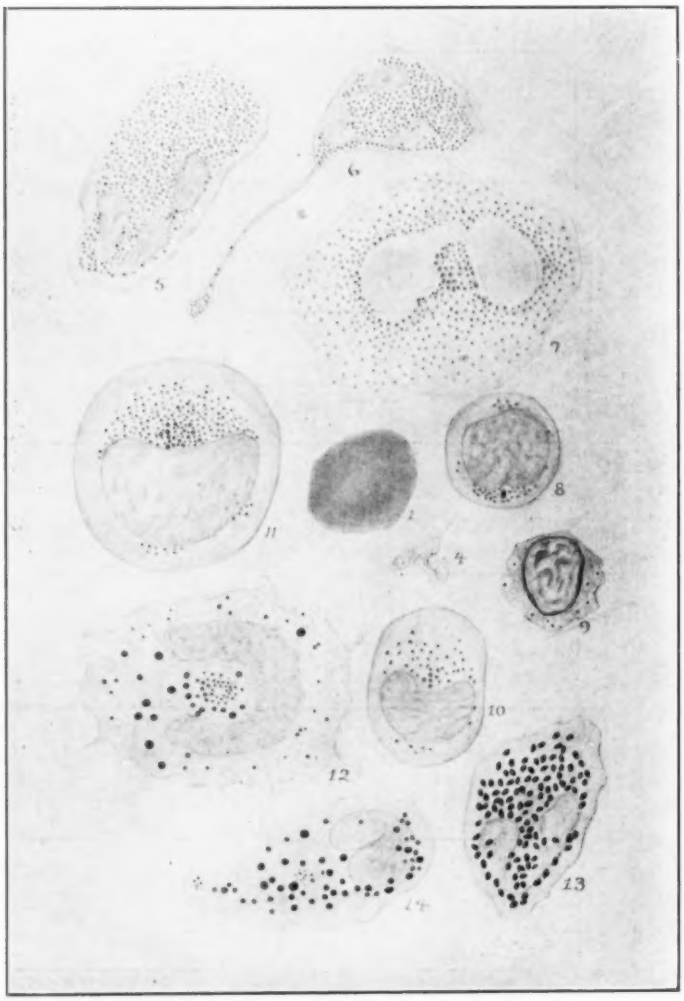

Plate I.—1, Erythrocyte: This cell is introduced for size contrast, the remaining cells being drawn to scale. Stained, it exhibits the straw yellow of hemoglobin with a faint greenish tint imparted by Janus green. *½ Platelet:* Multiangular in configuration, this element contains a few scattered granules which stain variously with Janus green and neutral red. *5, Polymorphonuclear Leucocyte:* The nucleus is observed to be dragging behind in the motile cell. Specific granules fill the endoplasm and when stained appear as small, round, refractile, yellowish pink bodies. A clear ectoplasmic rim is demonstrated. *6, Polymorphonuclear Leucocyte:* This cell exhibits a long, trailing pseudopod with a bulbous tip which may break off and resemble a platelet. *7, Nonmotile Leucocyte:* The nucleus is large, structureless, and evidently edematous. It is surrounded by many unstained, dark refractile granules which are scattered diffusely. *8. Small Lymphocyte:* There is a clear, homogeneous cytoplasm which contains typically clumped, short, rod-shaped mitochondria. A refractile "mahogany" granule appears opposite the shallow bay of the nucleus. *9, Effete Lymphocyte:* The nuclear membrane is fairly broad and refractile. Chromatin is coarse and smudgy. Hazy cytoplasm contains a few scattered, unstained refractile granules. *10, Intermediate Lymphocyte:* The clear cytoplasm contains many loosely clumped coccoid mitochondria among which may be found a few rounded neutral red bodies. The nucleus is kidney-shaped and its chromatin exhibits a fluted appearance. *11, Large Lymphocyte* (As seen in glandular fever): There is moderately coarse chromatin within the large bean-shaped nucleus. Mitochondria are clumped, and opposite the hof of the nucleus small neutral red bodies are arranged to form a pseudorosette. *12, Monocyte:* Small brick red bodies here form a true rosette within the nuclear hof. Peripheral to this aggregation are scattered numerous bodies with the same staining intensity but with varying size. The cytoplasm exhibits a ground glass appearance and has a foamy border with fragile, wavy pseudopodia. Tiny mitochrondria are scattered generally. The nucleus is horseshoe-shaped, has a wavy border, and its chromatin is loosely meshed. *13, Eosinophile:* The bilobate nucleus is observed dragging behind in the motile state. Its ectoplasm is clear and the endoplasm contains large, oat-shaped, yellow refractile granules. *14, Basophile:* Deep maroon, nonrefractile, round granules are seen scattered about the cytoplasm. They vary moderately in size and staining intensity. There are a few small clumps of tiny mitochondria. The bilobate nucleus is folded and seen at the fore end of the cell.

appear fewer in numbers, and for the most part as indefinite, nonrefractile, smudgelike, unstained bodies. Bone marrow studies have been the basis for the greater part of these observations.[12, 14, 34, 35]

The *Erythroblast* is approximately the same size as the previously described cell. It, too, as are all erythrogenic entities, is nonmotile. Its border is more distinct, and its nucleus, smaller in proportion to the amount of cytoplasm, is eccentrically placed. Mitosis is occasionally observed. There is increased chromatin density and the nucleolus is often obscured. The cytoplasm is entirely homogeneous and the yellow tint of hemoglobin is appreciably deepened. It is at this stage that red globules and their accompanying green blue shreds are first demonstrated. Other than these there is no structure stained with neutral red. Mitochondria are generally smaller, coccoid, and no longer diffusely scattered, but are loosely gathered about the nucleus, more often on one side. They now hold Janus green persistently.[12, 14, 34]

The *Normoblast* is very slightly larger than the mature cell. Its border is distinct and contains within its bounds a full complement of hemoglobin. The nucleus is now quite dense and even in the unstained state presents itself prominently. It is eccentrically placed and is observed to lie beside the cell membrane, often causing it to bulge. It may even be seen extruded into the surrounding medium. The cytoplasm is homogeneous and a significant percentage demonstrates the red globules described above. Mitochondria are much smaller in size and fewer in number. They are sharply localized to one side of the nucleus, and when this is finally lost to the cell, the clump of blue granules may remain visible for a short time.[14]

Leucocytes: The *Lymphocyte* is a vari-sized unit. Three forms are found in peripheral blood: the large, which is approximately 10 to 15 μ in diameter; a small one, approximately the size of a red blood cell; and a form intermediate between the two.[9, 45] The larger cell is, in the moist preparation, the less mature, according to Wiseman,[47] though it is generally believed that size is no conclusive criterion of age. Certainly, if the presence of neutral red bodies is any evidence of lymphocytic maturity, as some claim, size may not be utilized as such a standard. At all events, the *large lymphocyte* is a round, nonmotile cell with clear, glassy, homogeneous cytoplasm occasionally exhibiting a faint yellowish tint.[47] The nucleus, centrally or slightly eccentrically placed, is rounded or indented. It is quite large in proportion to the cytoplasm and contains a fairly thick meshwork of fine chromatin strands which do not aggregate peripherally or elsewhere. The mitochondria are short rods, relatively coarse, and tend to be clumped at one side, often opposite or overlying the hof of the nucleus. There are a few scattered chondriosomes in the paranuclear zone, and occasionally a small subsidiary clump opposite the main aggregation. Neutral red bodies may or may not be present. When they are, rarely exceeding 4 to 10 in number, they stain a deep red and appear as small, refractile globules about the same size as the mitochondria. They are scattered without pattern throughout the cytoplasm, often overlying the nucleus. Both Janus green and neutral red stained bodies may be observed to move with irregular activity within the stroma of the cell. In certain

states, such as infectious mononucleosis, wherein pathologic stimulation of the lymphocytopoietic organs obtains, many more neutral red bodies may appear and congregate in the region of the nuclear hof, displacing the mitochondria, and closely resembling the rosette of the monocyte.[21, 29, 31] Certain other criteria, as will be described elsewhere, serve to distinguish the cell.

The *Small Lymphocyte* is a rarely motile, rounded cell with a distinct border. It possesses a clear, untinted, homogeneous cytoplasm and a rounded, centrally placed nucleus. In a number of cells the nuclear contour is irregular and may even be fragmented, resembling the nucleus seen in the Rieder lymphocyte of the fixed smear.[25] In pathologic lymphocytic conditions the proportionate percentage of these forms increases. This is not so well demonstrated in fixed preparations. The chromatin network is denser than that of the previously described cell, the strands are quite coarse, and there is some condensation at the nuclear periphery. Mitochondria are fairly coarse and may be clumped at one side or encircle the nucleus with a single or double row. Neutral red bodies are rare but may occur up to 4 to 6 per cell.[9] This small form is the lymphocyte most frequently observed in normal blood.

The *Intermediate Lymphocyte* has a rounded or slightly irregular border which is definitely delimited. This cell, when stained with neutral red alone, has a characteristic motility. Its rate is slower than that of the polymorphonuclear, averaging about 4 to 15 μ per minute, and it progresses without pseudopod formation.[28] Its nucleus is found at the fore end of the moving cell, the cytoplasm and contents dragging behind and passing through static constriction rings (i.e., the rings retain their relative position to objects external to the cell while the cytoplasm passes through them, constricting as it does so and regaining its breadth afterward).[24] The cytoplasm has the clear, homogeneous consistency specific for the lymphocyte and is not tinted. The nucleus is relatively smaller in proportion to the cell bulk than in other mononuclear cells. It may be rounded, but is more often oval or kidney-shaped. Its chromatin content usually resembles that of the small form but may have a linear configuration which affords it a fluted appearance. There is, too, observed the "radkern" or wheel spoke arrangement as seen in the so-called plasma cell of the fixed smear. Mitochondria are similar in contour, size, and distribution to those of the other lymphocytes. Neutral red bodies likewise appear in this type but in a larger number of the cells than in the small form. They, too, may assume the pseudorosette appearance of the large lymphocyte.

There are not infrequently observed in blood preparations, particularly those from young children and patients with pronounced lymphocytosis, the "effete lymphocytes" described by Sabin.[18, 36] These present either a free nucleus or one surrounded by an ill-defined, hazy, slightly opaque remnant of cytoplasm. The nuclear contour is round and its border is sharp, thickened, and refractile. The chromatin is dense, smudgy, and vague in outline. External to the nuclear membrane either free in the plasma or imbedded within the cytoplasmic remnant are a few rounded, unstained, refractile bodies.

In some 34 per cent of small and intermediate lymphocytes, in rare monocytes, but in no other white blood cell there are observed one or a pair

of round, dark, mahogany colored, refractile bodies about 0.10 to 0.25 μ in diameter lying in the cytoplasm. Usually they occur opposite the bay of the nucleus. These bodies resemble morphologically certain of the cell inclusions described by Auer within large mononuclear cells in acute leucemia.[2] They have, however, been observed by the author in all bloods examined, their numbers varying in certain disease processes. Their significance is not at this time discerned. They are visible in the unstained cell but rarely persist in the fixed smear.

The *Lymphoblast*, about the size of the intermediate lymphocyte, is a rounded, nonmotile cell with relatively deficient, clear, yellowish cytoplasm.[9, 47] Its nucleus is small, round, and eccentrically placed. It possesses a fine, distinct nuclear membrane and a loose, thin meshwork of granular chromatin occasionally aggregated at the periphery, within which are found one or two darker nucleoli.[25] The mitochondria are short, plump, fairly coarse rods, which may be scattered throughout the cytoplasm, but more often assume a characteristic position close and adherent to the nucleus.[9] They are not clumped but tend to encircle the central structure. Neutral red bodies do not appear.

The *Polymorphonuclear Leucocyte* in the active state is an irregularly shaped cell about 12 to 23 by 6 to 10 μ with a distinct border which constantly changes its contour as a result of pseudopod formation.[23] The cell is rarely still but moves about in a circinate ameboid fashion. In the perfect preparation at body temperature its course is constant, in that for long periods of time the fore end remains persistently ahead. Frequently, as the cell proceeds, it may permit a long strand of cytoplasm to remain stretched out behind it. This process often has a rounded, somewhat bulbous tip. With continued movement the narrow pseudopod is withdrawn into the cell body, but occasionally its tip may break off and lie free in the plasma. It is these bits of protoplasm which have often been mistaken for motile platelets.

The rate of locomotion of the polynuclear cells has been estimated by McCutcheon to be approximately 34 μ per minute at 37° C. and this may increase moderately up to 40° C.[26, 27] Above this temperature the cell becomes static and there are alternately projected and retracted small ineffective pseudopodia. This is the so-called "prickle cell" of temperature paralysis although similar phenomena are noted at body temperature following incompatible transfusion.[36] Eventually, after swirling about seemingly wildly for a brief span, the cell will assume a rounded shape, all cytoplasmic movement will cease, and the nucleus will begin to stain, indicating cell death. At lower temperatures movement is much more sluggish, and below 15 to 20° C. the cell will be observed to "round up." Cell death will not, however, occur, for if after several hours the preparation is again brought to body temperature, normal appearance and movement will be resumed.[23, 27]

The polymorph nucleus has a fine, distinct border and a moderately coarse chromatin. It more often follows passively in the latter portion of the cytoplasm as the cell proceeds and may be elongated or compressed into all manner of bizarre shapes. The chromatin takes no dye normally. Staining of

nuclear structure is a definite indication for discarding the preparation, since cell death is imminent.[37] The cytoplasm is homogeneous and divided into definite endo- and ectoplasm.[5] The dividing line is well demarcated by the failure of neutral red bodies to proceed other than singly into the ectoplasm.

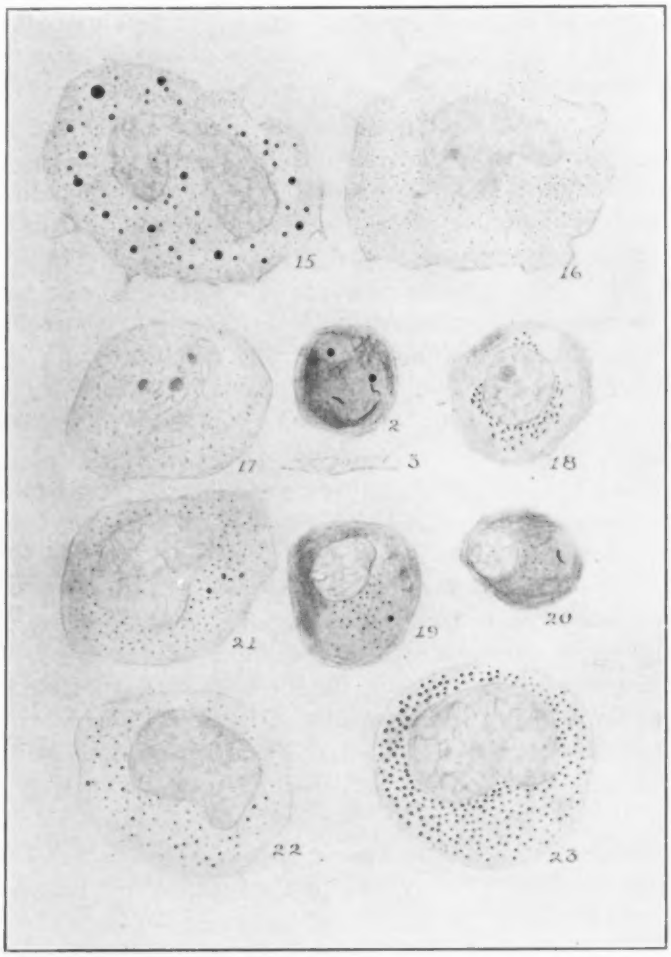

Plate 11.—2, Erythrocyte: This cell contains two bright red, small, refractile globules. There are also two finely granular, greenish shreds. *3, Platelet:* The substance of this element has a yellowish tint. There are a few fine, bluish green granules scattered within it. *15, Monocyte:* This is the usual appearance in normal human blood. It is similar to Cell No. 12 except that neutral red bodies are scattered without rosette formation. *16, Monoblast:* Blunt, rounded excrescences are seen on the cell surface. Chromatin is faint and fine, and the nucleus contains a single nucleolus. The cell is similar to Cells No. 12 and 15, but contains none of the structures stained with neutral red. *17, Myeloblast:* The nucleus has a thin, almost imperceptible nuclear membrane and contains a fine chromatin meshwork. There are three nucleoli present. The cytoplasm exhibits some mottling in the perinuclear zone and there are many thin, elongated mitochondria scattered diffusely. *18, Lymphoblast:* The cytoplasm is homogeneous and has a faint yellowish tint. The nucleus has a definite membrane, has an irregular chromatin mesh, and contains a single nucleolus. Coarse rod-shaped mitochondria are grouped close to the nuclear border. *19, Erythroblast:* The homogeneous cytoplasm of this cell has the straw yellow tint of hemoglobin. The nucleus is small and eccentric and mitochondria are grouped to one side. A superimposed, red globular body is seen. *20, Normoblast:* The nucleus appears at the periphery of the cell. Its chromatin is quite dense. The cytoplasm contains a full complement of hemoglobin and a few fine mitochondria clumped at one side of the nucleus. A small granular shred of greenish material is present. *21, Myelocyte "A":* Mitochondria are scattered diffusely and among them are a few neutral red bodies staining a brighter red than in the matured cell. *22, Myelocyte "B":* This cell is similar to No. 21 except that more neutral red bodies are present and mitochondria are fewer in numbers. *23, Myelocyte "C":* Here, there is present the full adult complement of neutral red bodies. Mitochondria are fewer in numbers and appear only at the cell periphery.

Streaming actively and dancing about within the endoplasm, which they fill completely, are numerous, tiny, round, yellowish pink, refractile granules which are specific for this polynuclear cell. These bodies rush about continuously in every direction and occasionally dart into the ectoplasm but are rapidly withdrawn again into the compact mass. Frequently they form the core of a pseudopod which is otherwise perfectly clear ectoplasm. In some of the neutrophiles large, nonrefractile vacuoles taking a deep red stain are observed among the streaming granules.[45] These usually appear within cells which have been in contact with the dye for one and a half hours or longer and may grow to the size of a nuclear lobe. Usually one appears, but there may be four or more. There are a few fine mitochondria present, but in the double-stained cell these are more often obscured by the other cytoplasmic contents.

The active leuocyte may not infrequently be observed to "round up" and remain still for a few seconds, but shortly it will again project pseudopodia and proceed on its course. In overstained preparations and near the edges where drying occurs this assumption of globular form is quite general and such areas are therefore significantly to be avoided. The author notes the number of these forms counted and utilizes their percentage as an indication of preparation perfection. These same drying regions, and occasionally freshly drawn blood, contain cells which have been termed by Sabin "nonmotile leucocytes."[36] She claims that at least in the rabbit these occur in cyclic showers throughout the day and may reach a level as high as 16 per cent of the total white blood count. This has not been conclusively confirmed in the human being. These cells exhibit large, swollen, structureless nuclei surrounded by scattered aggregations of unstained, slightly refractile granules. There are no well-defined delimiting membranes. No motility, other than some brownian movement of the granules, is observed.

The *Myeloblast* is an immobile, rounded cell with a thin, rather tenacious border. It is about 10 to 12 μ in diameter. The cytoplasm is rather hazy, somewhat mottled in appearance, and is evidently in a gel state, for no movement of its contents is noted. The nucleus is rounded or rarely indented, and it is eccentric in position. The nuclear membrane is smooth, even, and exceedingly thin, so thin, in fact, that it may be almost imperceptible.[15] Fine chromatin forms a loose meshwork with no condensation except about the nucleoli, which are two to four in number. Occasionally in subacute or acute leucemia, the nucleus may assume an irregularly indented contour which resembles the true Rieder cell. Indentations may progress to actual fragmentation of the nucleus. Mitosis is sometimes observed.[9] There are no neutral red bodies in this cell. Mitochondria are extremely fine, slender threads or commas and are diffusely scattered throughout the cytoplasm. They vary in size from cell to cell but within the same cell their size is quite constant. Rarely, they form a clump opposite one side of the nucleus.

Myelocytes are likewise immobile, rounded, and some 8 to 12 μ in diameter. They are subdivided into three arbitrary groups (A, B, and C), indicative of progressive maturity.[9] All contain a rounded or indented eccentric nucleus,

resembling closely that of the myeloblast, but losing the nucleoli as ripening occurs. The nuclear membrane, too, becomes more distinct. The cytoplasm is homogeneous and the mottling of the earlier stage is no longer observed. In the myelocyte "A" there are not more than 3 to 10 neutral red bodies. These are scattered without pattern throughout the mitochondria which still retain the same distribution as observed in the blast form. Occasionally they may clump in the region of the centrosphere, but this is rarely the case. These neutral red bodies are similar in shape, size, and refractility to the specific granules of the adult cell but stain with a deeper, bright red tint. The myelocyte "B" is like the "A" form except for an increase in the number of neutral red bodies. These, numbering 50 to 100 or more, are scattered throughout the mitochondria which now begin to decrease in numbers and are apparently being displaced toward the periphery of the cell. The myelocyte "C" contains a full complement of neutral red bodies which now fill the cytoplasm and compress the few remaining, minute mitochondria to the cell border. At this time fluidity occurs and movements of the cytoplasm ensue. Shortly afterward the nucleus indents further and ameboid movement is initiated so that the cell becomes the almost mature band form.

The *Monocyte* is a fairly large cell, 10 to 15 μ in diameter, barely, if at all, motile when stained with Janus green.[9, 17, 21] In preparations dyed with neutral red alone, it exhibits a slow, sliding motion, leaving one position entirely only after some five or ten minutes. Characteristically, it progresses by means of a rolling movement of the faint, irregular surface film which is followed more sluggishly by the deeper cell elements. Occasionally the cell appears to advance almost by shifting of cytoplasmic layers in successive depths. The border of the cell in the double-stained preparation is barely visible and irregularly broken by transient flat broad and spikelike protrusions. The latter wave about in the fluid currents and may either be withdrawn, break off, or merely disappear. Phagocytosis occurs by simple engulfment of débris and cell particles which lie in the neighborhood of the active monocyte. This cell apparently has a predilection for fragments rather than whole structures and rarely is an intact erythrocyte observed within it. Engulfed fragments are described as coming to lie characteristically at the outskirts of the rosette (vide infra).[37] As a matter of fact, such material as is phagocytized may be found in any portion of the cytoplasm.[21]

The protoplasm of the monocyte has a foamy, ground glass appearance with a mottled, indefinitely outlined surface film. It is evidently in sol form for enclosed bodies are observed to move about in a sluggish, jerky fashion, occasionally darting for a short distance. Streaming, as seen in the polynuclear cell, does not occur here. The nucleus is always eccentrically placed and may be rounded, indented, horseshoe-shaped or even resemble a petaled flower. In certain abnormal states fragmented or double nuclei may appear.[13] The nuclear membrane is fine, occasionally wavy, but quite distinct, and the chromatin content forms a very delicate, loose meshwork which affords a fine, sudsy appearance. Within the cytoplasm are observed numerous (40 to 80) neutral red bodies varying in size from minute dustlike granules to large

bulging vacuoles several μ in diameter. All these bodies take the neutral red stain with exactly the same intensity, a nonrefractile, fresh brick red.[44] The number of larger vacuoles varies directly with the age of the preparation. In the rabbit and guinea pig, but much less often in the human being, there has been described a characteristic development of small neutral red vacuoles of uniform size in concentric rows (2 to 5) about the clear area of the centrosphere opposite the nuclear hof. This has been termed the rosette.[4, 9, 20] The epithelioid cell of tuberculosis has been described by Sabin, Doan, and Forkner as developing from this form of monocyte by condensation and proliferation of the rosette vacuoles with diminution of their individual breadth.[38, 39, 41] As the rosette becomes larger and more compact, the cell enlarges, but no other significant morphologic change occurs. Scattered throughout the cytoplasm, peripheral to the rosette when it is present, are myriads of fine, dustlike coccoid mitochondria. These, along with the smaller neutral red bodies, tend to give to the cytoplasm its ground glass appearance.

The *Monoblast* is somewhat larger than the adult form and has no gross motility. Its border is fine, irregular, and exhibits many bleblike excrescences on its surface. There are fewer of the fragile spikelike protrusions. The cytoplasm is less foamy than that of the monocyte but the ground glass appearance is very similar. The eccentric nucleus is oval, indented, or occasionally horseshoe-shaped. Its chromatin is so poorly developed that its structure is hardly visible in the unstained state. Occasionally one or two nucleoli may be visualized. Small, blunt, coccoid mitochondria are abundantly scattered throughout the cytoplasm. Infrequently they form several small or one large clump in the nuclear indentation. In the early blast form there are no neutral red bodies, but as the cell progresses to the stage known as the premonocyte, some few neutral red bodies appear in the region of the centrosphere where they remain fixed. Their staining characteristics are similar to those of the neutral red bodies in the adult cell.[9]

The author has not identified *clasmatocytes* as such in the peripheral blood. They are reputedly large cells, 10 to 80 μ in diameter, actively motile in a manner similar to that of the monocyte.[30, 37, 39, 44] Carrel and Ebeling describe the cell as possessing an undulating membrane which acts as the propelling agent.[8] The cell is highly phagocytic and will engulf as many as twenty, thirty, or more whole red blood cells. Phagocytized objects characteristically come to lie adjacent to the nucleus initially.[37] The cell contour is relatively smooth but may exhibit transient bleblike and filiform pseudopodia.[48] The cytoplasm is abundant and evidently has no characteristic feature. The nucleus is round or oval, fairly large, and located near the center of the cell. Though large by contrast with the nuclei of other leucocytes, it is relatively small in proportion to the cell bulk. Indentation never progresses to the production of the horseshoe nucleus, but two or three nuclei have been observed in stimulated cells. The chromatin is very faint and is arranged in small scattered clumps. Neutral red bodies are present in large numbers, moving about constantly but exhibiting no regular distribution. There is no rosette. These bodies vary in size from dustlike particles to vacuoles the diameter of erythrocytes and in staining characteristics from bright scarlet to deep maroon.[37]

Unlike the vacuoles of the monocyte, this color is not constant but changes with intravacuolar activity. The clasmatocyte is supposed not to have mitrochondria, though some have been described, obscured for the most part by the neutral red bodies. A few unstained vacuoles may not infrequently be observed at the periphery of the cell.

The *Eosinophile* is of the same size as the polynuclear cell described and is actively motile in a similar manner. Its motility, however, is not as persistent, for after a preparation has stood for some thirty or forty-five minutes at body temperature, the cell becomes globular and its nucleus clear and structureless.[36, 45] The cytoplasm is homogeneous and exhibits an ectoplasmic rim into which the specific granules do not obtrude. The nucleus is usually bilobate and is dragged about, passively adjusting its shape to external stress. In the active state its chromatin content is somewhat similar to that of the neutrophile. The endoplasm is filled with streaming and dancing oval granules. These granules do not vary in size or color, are much larger than the specific bodies of the poly, and have a typical bright yellow, refractile appearance.

The *Basophile* is slightly smaller than the eosinophile and likewise has ameboid motion. Its motility, however, is almost as persistent as that of the polymorphonuclear cell, continuing for several hours. Its cytoplasm is quite clear and homogeneous, and does not exhibit division into endo- and ectoplasm. Its nucleus is round or lobate and has an irregular, shallow indented contour. Lobulation is often obscured by the tendency of the lobes to fold upon each other.[6] They do not stretch apart as do the nuclei of other polynuclear cells. Characteristically, when this cell is observed in the motile state the nucleus appears at its fore end, in a manner similar to the nucleus of the lymphocyte. The specific bodies of the basophile are relatively smaller and more sparse than those of the eosinophile, are round in shape, and exhibit no uniformity of size. They appear as nonrefractile, deep maroon globules which stream rapidly in the cytoplasm. Mitochondria are occasionally observed and appear as a small clump of tiny, green, coccoid granules.

The progenitor of the two latterly described cells is said to be the myeloblast. The intermediary forms, the myelocytes, develop exactly as do those of the neutrophilic series, except for the fact that the specific granules are similar to those of their respective adult forms.[9]

Platelets are small, faint, bodies, approximately 1.5 to 5 μ in diameter, possessing thin vague borders. Their contour is inconstant in that it may be globular, spicular, star-shaped, or grossly irregular. The spicular configuration is the most common form. The bodies are not motile and do not exhibit brownian movement when vital. Frequently, a bit of cytoplasm broken from a motile leucocyte may resemble a platelet and lead to the false conclusion that innate motility exists. No nucleus is observed. The cytoplasm is hazy and takes a greenish tint. Occasionally a few granules stained pink with neutral red may be observed. More often, however, the small granules present, scattered indiscriminately, are stained pale blue with Janus green. The platelet is notoriously fragile and demonstrates a marked clumping tendency. Having once clumped or become adherent to glass surface, it will disintegrate, discharge its content, and fade. The number in these preparations is much diminished at the end of one hour.

COMMENT

It is not the author's belief that the supravital technic may be utilized to the exclusion of the fixed smear. In combination with the examination of the fixed cells it offers further means of simplifying the identification of certain obscure cellular forms. The study of the living cell demonstrating specifically dyed structures offers additional aid in the differential diagnosis of blood diseases and appreciably broadens the bounds of hematologic research.[11, 16, 40, 46] It is with the belief that wider application should and could be made of this relatively simple procedure that this digest is offered. The simplicity of the method is stressed, for it entails no more difficulty, once the preliminary unfamiliarity has been eliminated, than any of the other established means of blood examination. In order to facilitate approach, a chart including the cardinal features of the cells commonly observed is appended.

SUMMARY

1. The development of the use of the supravital dyes is outlined briefly.

2. The method of preparation of the solutions of Janus green and neutral red for routine use is described.

3. The preliminary steps in preparation of glassware, warm box, etc., and a few precautionary measures to insure perfection of blood spreads are discussed.

4. Detailed descriptions of each of the cell forms observed in normal and pathologic blood are offered.

25 Bennet Street

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A COLORIMETRIC MICRODETERMINATION OF CHLORIDES IN BLOOD AND URINE*

T. V. Letonoff, M.S., Philadelphia, Pa.

RECENTLY, colorimetric microdetermination of chloride has been made feasible for the clinical laboratory by the marked gain in sensitivity over existing methods effected by the work of Westfall, Findley, and Richards.[1] In their procedure, based on that of Isaacs[2] and Emich,[3] chromate ion displaced from silver chromate by chloride is determined with the aid of the intense purple red color formed with symmetrical diphenylcarbazide. As the chromate liberated is proportional to the concentration of chloride, comparison of the color with that yielded by a standard solution of chloride enables calculation of the quantity of chloride in unknown solutions.

Certain features of the technic described by these authors interfere with its application as a practical clinical method. Thus the diphenylcarbazide reagent keeps only a few hours, while the color formed by the reaction with chromate fades to some extent after only three minutes. In the present paper preparation of a stable diphenylcarbazide solution is described. Furthermore, the permanence of the color given by chromate with this solution has been increased.

The test has been simplified further by substitution of a pulverized protein precipitant, zinc borate, in place of colloidal zinc hydroxide. This new precipitant has not been employed previously in blood analysis. Its use here is advantageous, particularly because it does not adsorb anions, while filtrates remain free from added reagents.

The technic described is suitable for analysis of 0.1 c.c. of blood or plasma. Blood obtained by puncture of the finger tip may be analyzed without difficulty by this method.

REAGENTS

Zinc borate powder is prepared by a method similar to that previously used in preparation of zinc hydroxide powder:[4] 34.8 gm. of powdered sodium borate, $Na_2B_4O_7.10H_2O$, are dissolved by warming in 800 c.c. of water and

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cooled. To it is added, with stirring, 20 gm. of zinc acetate, $Zn(C_2H_3O_2)_2 .2H_2O$, in 200 c.c. of water. After five minutes the precipitate is filtered by vacuum and washed with water until the washings give no test for chloride with silver nitrate. About 1 liter of water is required as the chemicals used have contained chlorides as an impurity. The wet material is dried in air at room temperature on a filter paper or porous plate. Drying is complete when the material can be powdered readily. It is ground to a powder in a mortar and stored in a stoppered bottle.

Silver Chromate.[2]—Add slowly 200 c.c. of a 5.5 per cent solution of potassium chromate to 100 c.c. of boiling solution of 10 per cent silver nitrate. Silver chromate settles out rapidly. Drops of the chromate solution are added until there is a slight excess of chromate which gives the solution a yellow color. After cooling, the silver chromate is thoroughly washed with distilled water and finally dried on a Buchner funnel. The silver chromate is spread in a thin layer on a glass plate and dried in a desiccator over sulphuric acid in the dark. When dry, store in a brown bottle.

Diphenylcarbazide, 0.02 per cent: Weigh 0.100 gm. of powdered symmetrical diphenylcarbazide. Transfer to a 1 liter beaker, add 500 c.c. of ammonia free water and cover with a watch glass. Dissolve by boiling for a few minutes. Cool and bring to 500 c.c. in a graduated cylinder. Preserve in a brown bottle. It is stable for at least two months.

Acetic Acid, 10 per cent: Accurately diluted.

Sodium Chloride Standard.—Weigh accurately 500 mg. of pure dry sodium chloride. Dissolve in water and dilute to 100 c.c. in a volumetric flask.

Working sodium chloride standard: Pipette 5 c.c. of the sodium chloride standard solution into a 100 c.c. volumetric flask and dilute to mark. 0.2 c.c. = 0.05 mg. NaCl.

METHOD

Into exactly 1.9 c.c. of water in a centrifuge tube, deliver 0.1 c.c. blood, plasma, serum or cerebrospinal fluid, rinsing the pipette with the water. Carefully calibrated Folin micropipettes are recommended. Add about 0.05 gm. of zinc borate powder. Stopper, shake hard for at least one-half minute and allow to stand for two minutes. Filter through a small paper.

When 0.8 c.c. to 1 c.c. has been collected in a centrifuge tube, add an excess of silver chromate and stir for about one minute, rubbing the solid against the wall of the tube with a stirring rod. Centrifuge and pour the supernatant liquid through a small filter paper. Similarly treat about 1 c.c. of the working standard sodium chloride with silver chromate. Centrifuge and filter. Use 0.2 c.c. of filtrate for a standard.

Transfer 0.2 c.c. of the chromate filtrates to clean, dry test tubes. Add 3 c.c. of the 10 per cent acetic acid. Add 10 c.c. of the 0.02 per cent diphenylcarbazide, mix by inversion and let stand ten minutes. Place some of the solution in the left colorimeter cup and read the standard in the right cup.

Calculation:

X = mg. NaCl per 100 c.c. blood
S = reading of standard
When unknown is set at 10 mm.
$X = 50 . S$

Urine: Prepare a 1 to 40 dilution of urine in water. Five hundredths cubic centimeter of urine made to a volume of 2 c.c. is sufficient for a determination, but more may be used if desired.

Shake about 2 c.c. of the diluted urine with about 0.05 gm. zinc borate powder and filter. Follow the technic outlined above.

Calculation:

X = gm. NaCl per 1,000 c.c. urine
S = reading of standard.
When unknown is set at 10 mm.
$X = S$

EXPERIMENTAL

Accurately diluted sodium chloride solutions were prepared covering the range from 200 to 800 mg. per 100 c.c. from which the chloride was precipitated by silver chromate and the color developed as described. When the series was read against a standard equivalent to 500 mg. per 100 c.c., direct proportionality between color and concentration was obtained throughout the entire range.

The color reached maximum intensity in about ten minutes, remaining constant for twenty minutes before beginning to fade. It is obvious that all water should be chloride free.

Because of the many advantages of a solid protein precipitant, an attempt was made to utilize the zinc hydroxide powder previously described by the writer.[4] However, small amounts of chloride were removed by this reagent. Other protein precipitants with the exception of colloidal zinc hydroxide also adsorbed chlorides, failed to remove uric acid, or interfered with the reaction. Powdered zinc borate was then tested and found to function effectively as a protein precipitant. This substance was inert with respect to chloride. Filtrates prepared by means of zinc borate powder gave no test for protein, zinc, or borate. Uric acid, which reacts with silver chromate, was completely removed. The filtrates were neutral.

When tested by analyzing normal and pathologic blood, plasma, serum, and cerebrospinal fluid and comparing with the Van Slyke[5] method, the variation between the two procedures was not greater than ±2 per cent. The chloride content of twenty urines determined by Harvey's[6] method and the diphenylcarbazide reaction likewise fell within these limits.

Analysis of protein-free filtrates after addition of pure sodium chloride to blood and plasma gave recoveries varying between 98 and 102 per cent.

SUMMARY

A simplified microprocedure for colorimetric determination of chloride in blood, plasma, and urine is described. The chloride content of 0.1 c.c. of blood or 0.05 c.c. of urine may be determined.

Powdered zinc borate is an effective precipitant of blood proteins that does not adsorb chloride.

The author wishes to extend his thanks to Dr. John G. Reinhold for assistance with the manuscript.

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THE ROSE BENGAL TEST OF LIVER FUNCTION: PHOTELOMETRIC METHOD*

W. P. Stowe, M.D., and G. D. Delprat, M.D., San Francisco, Calif.

IN PREVIOUS articles[1, 2] we have described a simple and satisfactory dye test of liver function. In the development of the photelometer by Sanford, Sheard, and Osterberg,[3] for the determination of color depth by the use of a photosensitive cell, it seemed that the last difficulty was overcome by the elimination of the personal equation in color matching. The following method has been evolved and used by us with satisfaction: The use of the normal plasma blank in the central cup of the photelometer corrects automatically for the yellow tint in icteric blood, heretofore difficult to match in colorimetric methods.

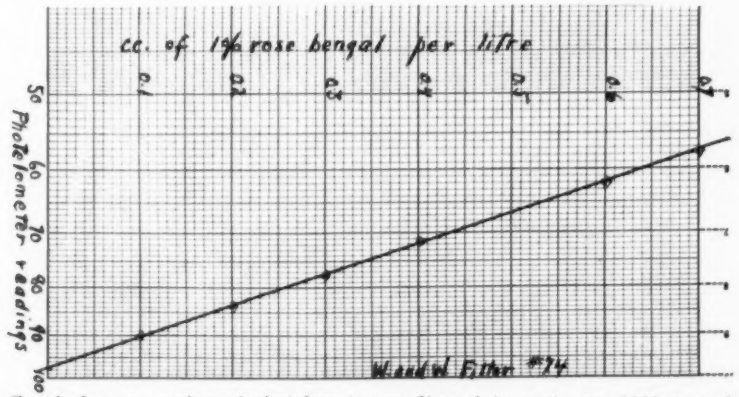

Fig. 1.—Graph for conversion of photelometer readings into parts per 1000 c.c. of solution.

The method is as follows: 10 c.c. of blood are withdrawn from an arm vein and oxalated at once (Sample A). Without withdrawing the needle, a syringe containing 10 c.c. of 1 per cent rose bengal solution in normal saline is connected and injected in about a ten-second interval. A fresh syringe filled with saline is substituted and the saline injected slowly during a two-minute interval when the second 10 c.c. blood specimen (Sample B) is removed and oxalated. Exactly six minutes from this time the third 10 c.c. specimen is removed (Sample C), either through the same needle or by separate venapuncture, and is oxalated.

All three samples are then centrifugated, the plasma removed, and the proteins precipitated with 2 c.c. of acetone per c.c. of plasma, followed by re-centrifugation until the supernatant extract is crystal clear. Treatment with Na OH to clear of lipoids and bilirubinate, as previously described,[2] may be desirable.

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Sample A is then placed in the central cup of the photelometer with W. & W. filter No. 74 in place, and the galvanometer needle brought to 100 through this solution. Samples B and C are then read on the photelometer and the readings translated into terms of cubic centimeters of 1 per cent rose bengal solution per liter by the accompanying graph (Fig. 1), plotted on Keuffel and Esser semilogarithmic coordinate paper No. 358-51. A similar curve for any individual photelometer and filter can be easily constructed from known dilutions of the rose bengal solution used.

The calculation previously suggested, $200 - \frac{200\ Rs}{Ru}$ = per cent of normal liver function, can be used if the parts per liter value of the two-minute sample (B) is substituted for Ru and the parts per liter value of the eight-minute sample (C) is substituted for Rs.

After extensive use we commend this method for accuracy and freedom from the errors of the "personal equation."

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St. Luke's Hospital
Valencia at 27th Street

A SIMPLE BASKET CARRIER FOR USE IN TISSUE DEHYDRATION*

Alvin J. Cox, Jr., M.D., San Francisco, Calif.

A NUMBER of methods have been suggested for transferring tissue blocks through dehydrating fluids in preparation for embedding. In the Stanford University Department of Pathology, the use of simple wire baskets in dehydration and paraffin infiltration of routine tissues has saved much time and has nearly eliminated the possibility of loss or mislabeling of specimens. The method has been in continuous use for more than one year in the preparation of 5,000 specimens for microscopic examination.

Although a basket similar to ours may be made by a craftsman, the following method provides a cheap, rapidly made, durable basket, which can be constructed by anyone with an old pair of scissors and a pair of pliers. The materials necessary are a piece of copper window screen 3½ by 2½ inches and a smaller piece about 1¼ inches square. Two vertical wires are removed from each end of the large piece (Fig. 1). This is then bent into approximately cylindrical shape, with the protruding ends of the horizontal wires directed inward so they interlace with those from the opposite side (Fig. 2).

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The ends of the uppermost wires are twisted about each other to hold the screen edges in position, and the joint is made more secure by weaving a piece of small copper wire back and forth as in Fig. 3. The screen is manipulated to form a cylinder, and two horizontal wires are removed from the lower end, leaving the ends of the vertical wires protruding. These are pushed through the small square piece of screen (Fig. 3). They are then bent toward the center and the protruding edges of the small piece of screen are trimmed off with scissors (Fig. 4).

The completed basket is approximately 1 inch in diameter and 2¼ inches tall. It can be placed completely within a wide-mouthed glass-stoppered bottle 2 inches in diameter and 4 inches tall. It is conveniently lifted from one bottle to another by means of a pair of forceps. Slices of tissue up to ⅞ inch in diameter are placed in the basket, with a strip of paper bearing the

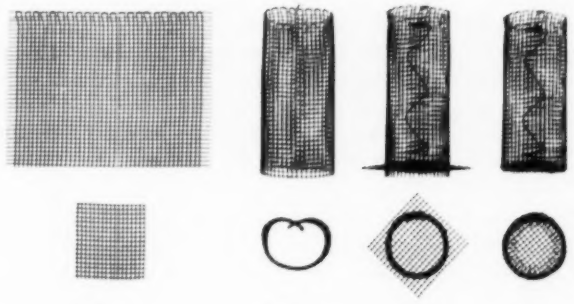

Fig. 1. Fig. 2. Fig. 3. Fig. 4.

Figs. 1 to 4.—Stages in construction of the basket carrier. Lateral and end views.

specimen number. The tissues are not handled again until after their final immersion in melted paraffin, when they are easily removed by inverting the basket while hot. The paper strip retaining the specimen number is permanently attached to the paraffin block in which the tissues are finally embedded. This avoids errors due to faulty copying of numbers. The basket has been found to interfere very little with dehydration or paraffin infiltration if it does not contain too much tissue. One basket will carry satisfactorily 2 or 3 large pieces, or 8 to 10 pieces, of tissue.

Fresh tissues may be fixed in the baskets, which are especially valuable for fixation of tissue curettings. These can be washed in running water without being removed. If the basket is placed in a shallow dish and the water stream directed into the top, the tissues are washed without danger of loss, since the water level within the basket does not rise above the edge of the dish.

Baskets which have been placed in melted paraffin may be cleaned easily by immersing them in waste xylene for a short period.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

POLIOMYELITIS, Human Convalescent Serum in the Treatment of Pre-Paralytic, Fischer, A. E. Am. J. Dis. Child. 48: 482, 1934.

Fischer analyzed data collected on two groups of patients gathered for the purpose of determining the value of convalescent serum in the treatment of preparalytic poliomyelitis. He does not consider that the question can be answered definitely because in spite of every effort to obtain comparable data, it was not possible to do so. It has been pointed out that it is particularly difficult in the case of medical material to obtain strictly similar groups. The most important point which the study has emphasized is that 579 patients with poliomyelitis were seen early in the course of the disease, of whom 477 were treated by injection of convalescent serum and 102 were untreated. He showed that the sex, age, stage of the epidemic, day of treatment, dosage and method of administration of serum had no influence on the outcome of the disease so far as can be determined statistically, while, on the other hand, the temperature, cell count and general clinical impression could at times be used as an indication of the degree of severity of the illness.

This was the first epidemic in which an attempt was made to evaluate the serum fairly not only by treating patients in the preparalytic stage but also by examining and following a number of control patients. The author has demonstrated that the outcome for the treated patients was no better, if as good, as for the untreated ones; while the controls were probably somewhat milder, he feels that no advantage was shown by the other group. For this reason it should be possible in the future to obtain a sufficient number of control cases, for the present study has demonstrated that there is no proof that a physician is depriving his patient of an equal chance for complete recovery by not administering convalescent serum. Lacking a substitute therapeutic procedure, it would seem fair in subsequent epidemics to retest the value of potent serum in the preparalytic or meningitic stage of poliomyelitis, using, if possible, exactly parallel or alternate cases for control. If this is impossible, at least comparisons should be made as to the outcome for treated and untreated patients, using as a basis the day of the disease, the symptoms and other factors such as those discussed in this report.

MENINGOCOCCUS, Precipitation Reactions of, With Immune Serums in Agar Plates in Relation to Antigenic Activity, Kirkbride, M. B., and Cohen, S. M. Am. J. Hyg. 20: 444, 1934.

Significant supplementary data on the serologic and immunologic properties and activities of the standard meningococcus strains used for many years in serum production, were obtained from studies of the precipitation reactions associated with the growth of the cultures on immune serum agar plates. At the same time, similar studies were made of a number of other strains, both recently isolated and stock. The results were, in general, in accord with previous laboratory findings which had failed to demonstrate any marked superiority in the new as compared with the older strains. Besides the laboratory observations, convincing proof of the present value of the standard strains as antigens was established by the continued favorable clinical reports on the use of the homologous therapeutic serum. Evidence was also obtained indicating the importance of maintaining the cultures used in serum production under the most favorable environmental conditions, and, conversely, the possible harmful effect on cell capacities of apparently slight alterations in these conditions and, hence, on the active principles of the serum itself. Certain of the observations made may be enumerated briefly as follows:

1. Meningococcus strains of Groups I and III isolated more than fifteen years previously were found to have retained their activity in elaborating the specific precipitating substance as determined by the immune serum agar plate test. These strains, also, produced, both in horses and in rabbits, serum of precipitative activity.

2. Strains of Group II and those of group "X," related to Group II or "IV," lacked, in general, demonstrable specific precipitating substance, and produced in animals serums of little or no precipitative activity.

3. Differences in the precipitation reactions of the cultures were not found to be related primarily to the interval since isolation and the period of artificial cultivation. Both stock and recent strains of Groups I and III gave rise to well-marked haloes. Neither stock nor recent strains of Group II were found to possess similar precipitative activity.

4. Substitution of certain semifluid or broth media for the dextrose serum agar used in the maintenance of cultures resulted, in the case of several Groups I and III strains, in the reduction or loss of the specific precipitating substance, and also, in the case of one strain, of the precipitinogenic properties. These results suggest that, in addition to the selection of representative strains, the medium used in their maintenance may play an important rôle in serum production.

5. Marked differences were observed in the precipitative activity of therapeutic serums produced by different laboratories.

TYPHOID, Study of Production of Somatic and Flagellar Agglutinins in Response to Antityphoid-Paratyphoid Inoculations, Dennis, E. W., and Berberian, D. A. Am. J. Hyg. 20: 469, 1934.

The serums of 114 volunteers, including individuals who had had enteric fever with and without subsequent vaccination, individuals who had no history of enterica or of vaccination, and individuals who had been vaccinated from one to ten times, were submitted to a quantitative receptor analysis before and after vaccination.

A comparative study was made of the influence of formolized, phenolized, and heat-killed vaccines prepared in our laboratory, and four commercial antityphoid preparations on the production of agglutinins.

All of the vaccines used induced the production of "O" agglutinins in considerable quantities. The vaccines prepared in the authors' laboratory of recently isolated, smooth, virulent strains gave rise to "O" titers comparable with those seen in active enteric infection; and "O" titer of 1:1,200 for *Bact. paratyphosus A* was recorded. The formolized vaccine was the most efficient in stimulating "O" agglutinins.

The vaccines prepared in their laboratory were 200 per cent more efficient than the commercial products in the production of "O" agglutinins.

There was no correlation between the number of previous vaccinations, the interval of time since the last vaccination, and the height of the titer, making the establishment of an arbitrary diagnostic titer impossible. Hence, a single qualitative receptor analysis is not capable of differentiating between inoculation agglutinins and those due to infection.

Twenty-five per cent of inoculated individuals lose all traces of "O" agglutinins within a year; more frequent inoculation is indicated.

BILIRUBIN, BLOOD: Ernst-Foster Method for, Gadjos, A. Rev. Med-Chir. des Malad. du Foie, Paris 9: 45, 1934.

Gadjos reports this method as superior to the van den Bergh for measuring weak bilirubinemia.

1. To 1 c.c. of serum add 2 c.c. of acetone.

2. Filter, centrifuge, and compare with standard.

The standard is a 1:6,000 solution of potassium bichromate representing 0.329 mg. per cent of bilirubin in distilled water. Comparison is made either in the colorimeter or with a series of graded standard tubes. The reading is multiplied by 3. If the bilirubin concentration is low, only 1.5 c.c. of acetone is added to the serum and the reading multiplied by 2.5.

ANEMIA, Iron-Deficiency, Diagnosis and Treatment of, Bethell, F. H., Goldhamer, S. M., Isaacs, R., and Sturgis, C. C. J. A. M. A. 103: 797, 1934.

Iron-deficiency anemia results from a lack of sufficient available iron for normal hemoglobin formation. Such a lack may be induced by (1) depletion of the iron reserves from continued loss of blood, (2) inadequate intake of food iron and (3) improper absorption of the element from the alimentary tract and, as a rare possibility, (4) from inability to utilize available iron.

In women with achlorhydria, anemia may develop as the result of the physiologic loss of blood. In such cases a "conditioned deficiency" dependent on the lack of hydrochloric acid may be said to exist.

The clinical features presented by patients with iron deficiency anemia are not specific. They include the effects of lack of hemoglobin supplemented by the manifestations of whatever associated condition may be present. By contrast, the blood in such patients possesses certain definite characteristics that are of diagnostic value. The relative decrease of hemoglobin exceeds that of the erythrocytes, and the average size of the red corpuscles is reduced, although proportionately to a less extent than the diminution of hemoglobin. Consequently, the color index and the mean erythrocyte diameter, volume, hemoglobin and hemoglobin concentration are below normal.

The effects of treatment with simple iron preparations of forty-two cases of iron-deficiency anemia, twenty-eight with achlorhydria, are shown in tabulated form. These results compare favorably with those reported by others employing combinations of iron with other substances in the treatment of the same type of anemia.

Relatively large amounts of ingested iron are required for satisfactory clinical and hematologic improvement. Ferrum reductum, 1.5 gm. daily, or ferric ammonium citrate, 4 gm. daily, administered in three divided doses after meals, in our experience, is therapeutically optimal. Following the institution of treatment a latent period, during which no change in the peripheral blood picture occurs, is attributed to the time required for maturation by the primitive erythrocytes in the bone marrow. In general, the blood of patients with acid gastric secretion responds more promptly to iron medication, and a smaller dosage of the element is required than is the case of those with achlorhydria. In both groups the erythrocyte and hemoglobin values are usually restored to normal after from six to eight weeks of therapy. Patients with achlorhydria often require continued treatment with iron in order to prevent recurrence of anemia.

Administration of highly purified ferrum reductum in conjunction with a "low copper" diet did not detract from the efficacy of the iron, as evidenced by the rate of hemoglobin formation.

HEPATIC CIRRHOSIS: The Nonglucose Reducing Bodies in Blood and Their Variation in Sugar Tolerance Tests, With Special Reference to, Menon, V. K. N., and Rao, M. V. R. Ind. J. M. Res. 22: 29, 1934.

The nonglucose reducing bodies in blood in health and disease, with special reference to cirrhosis of the liver, were studied, and their variations in sugar tolerance tests determined. It is pointed out that:

The nonglucose reducing bodies vary within wide limits in health and disease.

The average values for "glucid X" and "y-reduction" in cases of cirrhosis of the liver do not show much variation compared to the normal standards obtained in this series. The glutathione content of the blood, however, is diminished in these cases of cirrhosis of the liver.

In most of the sugar tolerance tests in health and disease, "glucid X" and glutathione vary in the same direction as the blood sugar, while the y reduction shows a considerable fall (most marked at the end of one hour) with the rise in the blood sugar. In some cases the y-reduction actually becomes negative.

From the data at present available the nonglucose reducing bodies in blood do not seem to be in any way responsible for the low fasting blood sugar found in cases of cirrhosis of the liver.

The glutathione of the blood cannot be considered to be mainly responsible for the "y-reduction."

A study of the ascorbic acid (vitamin C) content of the blood and its variations in sugar tolerance tests in these cases may throw further light on the nature of the "non-glucose reducing bodies" present in blood.

SILICOSIS, Pulmonary Lesions Experimentally Produced By Intratracheal Introduction of Aluminum Oxide and Borosilicate-Glass, Lemon, W. S., and Higgins, G. M. Am. Rev. Tuberc. 30: 548, 1934.

Very fine particles of aluminum oxide and of borosilicate-glass, of a size permitting phagocytosis, were introduced through a tracheal cannula into the lungs of two series of rabbits, and the animals were permitted to live for from one to twenty weeks.

The particles of each material produced an immediate inflammatory reaction, characterized by escape into the pulmonary tissues of serum, erythrocytes, and polymorphonuclear cells. At the same time, phagocytosis of particles occurred, and the alveolar phagocyte was the most active and important phagocytic cell. Its efficiency as a phagocyte was evidenced by the very large numbers of ingested particles. They were often so closely massed, and so numerous that they obscured the cytoplasmic detail of the cell, distorting the contour or rupturing the boundary of the cell.

Even in the experiments of shortest duration, there was evidence of migration of phagocytic cells, and of exclusion of them from the lung by way of the lymphatic structures and bronchi. The latter method of exclusion was indicated by the exudates containing phagocytic cells laden with crystals, in all of the divisions of the airway, from the alveoli themselves to the larger bronchi, the cilia of which were effective instruments in the process of removal. The former method of exclusion was indicated by finding test material in the tracheobronchial lymph nodes, and by finding evidences of agglutination of phagocytes laden with crystals in all parts of the lung where lymphatic tissue abounds.

Throughout all the experiments, the lesions which appeared as miliary or confluent, roughly spherical nodules were found in the peribronchial, perivascular and subpleural lymphatic distributions. They were conglomerate and largest when situated at the hilum, and smallest and most discrete when related to smaller bronchi or vessels.

When aluminum oxide was used, neither the histiocytes nor the fibroblasts were stimulated to activity. The lesions were made up only of aggregations of phagocytic alveolar histiocytes, which gave no evidence of multiplication nor of transformation into fibroblasts. When borosilicate glass was used, the lesions found after the longest period showed some evidence of replacement by diffuse fibrosis having taken place in the stroma of the lung. The earlier lesions were almost identical to those resulting from aluminum oxide.

There was no instance of a lesion, produced either by aluminum oxide or by borosilicate glass, that even remotely resembled those produced by silica when particles of equal size, of uncombined silicon dioxide, were similarly introduced into the lungs of rabbits. There was no evidence that either material circulated in the blood stream, and accumulation of phagocytic cells bearing crystals was not found in remote organs.

These observations seem to support the theory of solubility, of Gye and his associates, who have expressed the belief that the characteristic connective tissue nodules of silica are produced because a tissue poison results from the contact of silica with the pulmonary tissues. The authors' experiments suggest that the biologic activity of uncombined silica is absent when borosilicate glass or aluminum oxide is employed and characteristic hyperplasia does not occur.

TUBERCULOSIS, Intestinal in 1,400 Autopsies, Crawford, P. M., and Sawyer, H. P. Am. Rev. Tuberc. 30: 568, 1934.

In this series, 68.8 per cent of cases of fatal phthisis showed at autopsy ulcerative tuberculous lesions of the intestines.

Ulcerative intestinal tuberculosis was found as a complication of tuberculous pulmonary disease in 87.5 per cent in the colored race, as against 42.2 per cent in whites.

Tuberculous laryngitis occurred in 36.6 per cent of cases of intestinal tuberculosis, but 96.6 per cent of cases of tuberculous laryngitis showed intestinal ulceration.

Only 10.8 per cent of the ulcers in this series were of the classical girdling type.

In 540 cases of intestinal tuberculosis complicating tuberculous pulmonary disease, 53.7 per cent occurred in cases whose pulmonary symptoms had a duration of six months to three years. In 34 per cent of cases the intestinal lesions were asymptomatic, and in 35 per cent the duration of enteric symptoms was less than six months. No cases, showing preulcerative lesions only, had marked intestinal symptoms.

There were 51.6 per cent of cases classified as having had good treatment, as against 36.8 per cent of cases showing evidence of serious deficiencies in treatment.

Some form of collapse therapy for the control of the pulmonary disease had been carried out in 20 per cent of the cases.

Intestinal tuberculous ulceration is found in a majority of cases of fatal phthisis.

The portal of entry for tuberculous intestinal infection appears to be the lower ileum.

In cases of fatal phthisis with intestinal ulceration the incidence of amyloid disease is only slightly higher than in those without this complication.

A large proportion of tuberculous intestinal ulceration is not characterized by any definite symptoms during life.

The clinical detection of late tuberculous enteritis is of prognostic importance only; it portends an early fatal termination of the case.

TUBERCULOSIS: Large Doses of Tuberculin in Testing Guinea Pigs Inoculated for Diagnostic Purposes, Magath, T. B. Am. J. M. Sc. **188**: 403, 1934.

When testing clinical material for the presence of Mycobacterium tuberculosis by animal inoculation, a pair of guinea pigs should be inoculated, one subcutaneously and the other intraperitoneally. In order to hasten the test, one or both animals should be tested at the end of four weeks with 0.5 c.c. OT tuberculin injected subcutaneously.

More than three-fourths of the positive animals will be killed within forty-eight hours and the diagnosis hastened by three weeks. The chances that any other given animal will later prove to have lesions of tuberculosis is one in thirty.

EOSINOPHILIA, The Effects of Vaccines and Bacterial and Parasitic Infections On, In Trichinous Animals, Spink, W. W. Arch. Int. Med. **54**: 805, 1934.

The number of circulating eosinophile leucocytes in animals infected with *Trichinella spiralis* was reduced following infection with *B. tuberculosis, Staph. aureus* and *Trypanosoma equiperdum*. Animals which had received repeated injections of typhoid vaccine responded with a rise in the eosinophile level. No change was noted following the injection of heat-killed tubercle bacilli.

Studies of the bone marrows from the same animals did not reveal a corresponding decrease in the number of eosinophile cells.

Trichinous animals having a superimposed infection of tuberculosis or trypanosomiasis had less reaction around the encysted parasites in the muscles than the control animals. Trichinous animals inoculated with typhoid vaccine showed similar changes in the muscles.

Trichinous animals subjected to a high level of dry heat responded with an absolute rise in the circulating eosinophilic leucocytes.

No relationship was found between the weights of animals and the level of eosinophilic leucocytes in the peripheral blood.

The number of circulating eosinophile cells did not appear to be related to the mode of encystment of *Trichinella spiralis* in the muscle.

HAVERHILL FEVER (Erythema Arthriticum Epdemicium), Place, E. H., and Sutton, L. E. Arch. Int. Med. 54: 659, 1934.

An epidemic of a disease, apparently a new clinical entity, named epidemic arthritic erythema or Haverhill fever, is described. It is characterized by an abrupt onset, often with a chill, a rubellaform to morbiliform eruption, often scanty, chiefly on the extremities, with a tendency toward hemorrhage into the lesions, and an inflammation of the joints with marked pain and tenderness not infrequently of prolonged duration.

The disease occurred as a markedly localized epidemic and was undoubtedly spread through the raw milk supply.

An organism, described only once before, is believed to be the cause, having been found in the blood stream in 11 of the 17 cases in which the blood was cultured and in the fluid of the joint in the 2 cases in which the fluid was cultured.

Agglutinins were present in the blood of the infected persons but absent in the controls.

Cutaneous reactions to killed suspensions were present in 83 per cent of the patients tested late in convalescence, although absent in the controls.

Although crippling may be marked for a time, recovery tends to occur in from one to two months, with a small number of patients having persistent joint symptoms. No fatalities occurred.

TUBERCLE BACILLUS, Cultivation of, From the Blood Stream by Loewenstein's Method, Maier, E. Am. Rev. Tuberc. 30: 695, 1934.

In 198 cases of advanced pulmonary tuberculosis, attempts to cultivate the bacillus from the blood stream by the Loewenstein method gave negative results.

LYMPHOGRANULOMA INGUINALE, The Frei Test in, Recovery of Antigen From a Pustular Eruption, Strauss, M. J., and Howard, M. E. J. A. M. A. 123: 1830, 1934.

From a small series of experiments the authors conclude that:

1. If a Frei reaction is strong enough to cause vesicle formation, the contents of this vesicle are capable of producing a reaction similar to the Frei reaction in patients with lymphogranuloma inguinale.

2. The substance causing this reaction is probably a remainder of the Frei antigen originally injected and not combined with antibodies.

3. These antibodies are not circulating antibodies but fixed antibodies.

It is realized that this brief experiment is not conclusive but they feel that it is expedient to publish the results so that they may be repeated by others who have more material at their disposal and that further experiments which theirs suggest may be undertaken.

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EDITORIAL

The Treatment of Syphilis

IN AN epidemiologic study of influenza the writer in 1920 suggested the organization of an international centralized bureau for epidemiologic and similar studies of the problems of infectious disease. That others were thinking along similar lines at that time was soon evidenced by the inauguration of the Health Organization of the League of Nations.

Although the League of Nations may be impotent to cope with the problems of warfare among nations, at least one splendid advance may be credited to it, namely, its Health Organization. The situation is very similar to that accompanying the origin of the Red Cross in which doctors and scientists from all nations have succeeded reasonably well in collaboration for the good of mankind, while the nations themselves continue at each other's throats.

One of the problems which has been studied in great detail by the Organization has been that of syphilis. Much of the earlier work has been devoted

to standardization of diagnostic methods. The March, 1925, *Quarterly Bulletin* presents a discussion of the results of treatment of this disease, with collaborative effort from several countries. The countries represented are Denmark, France, Germany, Great Britain, and the United States. An idea of the standing of the collaborators in the various countries may be had from the names of those who have represented the United States: John H. Stokes, Udo J. Wile, Paul A. O'Leary, J. Earle Moore, H. N. Cole, and several members of the Public Health Service.

Conclusions drawn from the study are based on a total of 25,623 cases, approximately three-fifths men and two-fifths women. From the study, a program of ideal treatment has been elaborated, which we might term the League of Nations Health Organization Program. Since, if such a program is to be followed by the individual physician, he should know it in detail, and since this program is not generally available in the literature of this country, we herewith reproduce it in full as outlined by the Organization.

1. Treatment should be recommended as early as possible in the seronegative primary stage. In this connection, the fullest possible use should be made, for purposes of diagnosis, of the microscopic examination of secretions from primary lesions or from lymph glands.

2. It should be emphasized that, prior to the institution of either of the systems of treatment outlined below, there should be an adequate physical examination to determine the absence or otherwise of any indication for caution in respect to the dosage.

3. It is essential that, in carrying out the treatment, a strict supervision of the patient be exercised, especially in respect of mucous membranes, skin, kidneys and liver.

4. Observation, clinical and serologic, after completion of treatment should be adequate and in any case for not less than three years.

5. Adequate examination of the spinal fluid, at least before dismissal from observation, is essential.

6. The principles to be followed in carrying out the actual treatment should be as follows:

(a) To employ a comparatively heavy individual dosage of the arsenobenzene and of the bismuth or mercurial compounds, the doses being administered in comparatively rapid succession, especially at the commencement;

(b) To maintain a persistent attack on the disease, avoiding intervals of such length as to afford the parasite an opportunity of recovering;

(c) To administer approximately as much treatment to primary as to secondary cases.

7. The material studied does not enable a clear decision to be made as to the relative merits of intermittent treatment with courses of injections in rapid succession separated by rest intervals of some weeks and continuous treatment as defined on pages 149 and 150 of Professor Martenstein's report, or between the simultaneous employment of both arsenical and bismuth or mercury, and the system in which bismuth and mercury are withheld until a number of arsenical injections have been administered.

Nevertheless, it seems practicable, from the results of the analysis and from the personal experience of the Experts, to formulate a system of intermittent treatment and one of continuous treatment, either of which can be expected to yield satisfactory results in ordinary cases or early syphilis.

It seems possible that the intermittent treatment which is suggested below may in effect be continuous, or practically continuous, treatment, owing to the continued absorption of bismuth from the sites of the injection for some weeks after any temporary suspension of the treatment.

PLAN OF INTERMITTENT TREATMENT

1. Males

For adult males of average weight aged less than 50 years and in whom there is no contraindication, a number of courses of injections on the plan described below. It should be said that, at the beginning of this course, some administer at once the full weekly dose (0.60 gm. to 0.75 gm.), while others divide it into two doses (e.g., 0.30 gm. and 0.45 gm.) so far as the first week is concerned.

WEEK	"914"	"606"	INSOLUBLE COMPOUND OF BISMUTH* CONTAINING BI-METAL
1st	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
2nd	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
3rd	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
4th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
5th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
6th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
7th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
8th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
9th	----------	----------	0.20 to 0.24 gm.
10th	----------	----------	0.20 to 0.24 gm.

*(1) By *insoluble* bismuth is here meant compounds of a very slight solubility in water. They should therefore be given in suspension—those of extremely slight solubility (the oxychloride, etc.) usually in a watery suspension, those that are more soluble (the subsalicylate, the iodo-bismuthate of quinine, the alkaline tartrates, etc.) suspended in a vegetable oil. If a *liposoluble* compound (*e.g.*, the camphocarboxylate, etc.) is preferred, it is desirable that the injection be given twice weekly in half doses.

The dosage of all bismuth compounds should be calculated according to their content in bismuth metal.

(2) As an alternative to bismuth, a course of mercury may be given, either in the form of inunctions (forty days at 3 gm. of *Unguentum hydrargyri*) or of injections (70 mg. calomel or 120 mg. salicylate of mercury, etc., suspended in a suitable base).

It is recommended that:

(a) In cases which remain or become serologically negative during, or by the end of, the first course, four such courses be administered, with intervals of three to five weeks between any two courses.

(b) In cases which have not become seronegative by the end of the first course, in addition to the amount of treatment shown in (a), further courses should be administered until the patient has received as a minimum three beyond that which has ended with negative serum reactions. At the option of the individual clinician, this treatment may be prolonged as may be considered necessary.

(c) Cases presenting signs of clinical relapse of an early type should be dealt with on principles similar to those enunciated in (b).

2. Females

For females (nonpregnant), treatment should be administered on the plan outlined for males, with the exception that the single dose of "914" should be reduced by 0.15 gm. and that of "606" by 0.1 gm.

In the event of any reduction in the amount of treatment being indicated, it is recommended that this be effected by reducing the number of arsenical injections rather than by reducing the individual dose or increasing the interval.

As an optional scheme more in harmony with the trend toward longer courses, three series of ten to twelve injections each of the arsenical drugs may be given. To secure an overlapping of the heavy metal and the arsenical, believed by some observers to protect against neurorelapses, begin the bismuth two, three, or even four injections before the end of the longer arsenical course, continue it through the period in which the arsenical is suspended, and on into the beginning of the next arsenical course. The bismuth is then suspended while the arsenical course is completed.

PLAN OF ALTERNATING CONTINUOUS TREATMENT FOR EARLY SYPHILIS

DAY OR WEEK	"606"	INTERIM TREATMENT	SEROL. TEST	REMARKS
Day				
1	0.3-0.6	----------	1	"606" dosage for first three injections at level of 0.1 gm. for each 25 pounds (11.3 kg.) body weight. Average subsequent dosage, 0.4 gm., men; 0.3 gm., women, the fourth and subsequent injections in the first course at weekly intervals. In average patient, all lesions heal rapidly and blood serologic reaction becomes negative during first course. If "606" cannot be used, substitute 8 to 10 doses 0.3 gm. silver arsphenamine (silver salvarsan, silver arsenobenzol, etc.) or 10 to 12 doses "914" (0.45-0.6 gm. maximum for women and 0.6-0.75 gm. for men). This applies also to subsequent courses.
5	0.3-0.6			
10	0.3-0.6			
Week				
3	0.4			
4	0.4			
5	0.4			
6	0.4			
7	0.4	----------	1	If mercury is used, note overlap of one week at end of first and start of second "606" courses. At this point, a few days without treatment may be dangerous. Neuro-relapse.
8	---------	Bismuth, 4 doses,		
9		0.2 gm. and		
10		K. I., or Ungt.		
11		Hg. and K. I.		
12	0.4	----------	1	"606" starts, bismuth stops. Watch for provocative serologic reaction after first dose of "606."
13	0.4	----------	1	
14	0.4	----------	-------	Try to prevent short lapses in treatment, especially at this early stage.
15	0.4			
16	0.4			
17	0.4	----------	1	
18-23	---------	Bismuth, 6 doses (or Ungt. Hg.) and K. I.	-------	Bismuth is better than mercury. Use it if possible. Examine cerebrospinal fluid if patient's cooperation can be secured at about this time. If found to be abnormal, continue or intensify treatment as required, re-examining fluid within six months.
24	0.4			
25	0.4			
26	0.4			
27	0.4			
28	0.4			
29	0.4	Bismuth, 8 doses	-------	
30-37	---------	(or Hg.) and K. I.		
38	0.4	----------	1	
39	0.4			
40	0.4			
41	0.4			
42	0.4			
43	0.4	----------	1	
44-53	---------	Bismuth, 10 doses (or Ungt. Hg.) and K. I.	-------	Note that bismuth or mercury courses are gradually getting longer—four, six, eight and now ten weeks.
54	0.4	----------	1	
55	0.4	----------	-------	The average seronegative, seropositive primary or early secondary patient should have at least five courses of "606."
56	0.4			
57	0.4			
58	0.4			
59	0.4	----------	1	
60-69	---------	Bismuth, 10 doses (or Ungt. Hg.) and K. I.	-------	It is safer to finish treatment with bismuth or mercury rather than with "606."
70-122	Probation	No treatment ----	6-12	
123	Complete physical and neurologic examination, lumbar punctures, and, if possible, fluoroscopic examination of heart and great vessels.			

The bismuth salt advised for this system is bismuth salicylate in oil suspension, in full adult dosage with due regard for weight. Other preparations of bismuth may be used only with due regard for an equivalent metallic content and for their rate of elimination. The mercurial inunction is 50 per cent metallic mercury in a suitable fatty base, dose 4 gm. per inunction, five to six inunctions per week. The use of the iodide is optional, depending on indications.

The use of insoluble mercurials intramuscularly in this system is not recommended.

It should be further understood that, when heavy metal is employed after the last "606" course, the heavy metal courses are to be separated by rest intervals of six to eight weeks between each series of ten weeks' injections, or each course of forty inunctions.

In cases of primary syphilis which have remained seronegative throughout, a minimum of 5 courses of "606" or "914" should be given. Cases of seropositive primary syphilis should receive the full treatment called for by this system.

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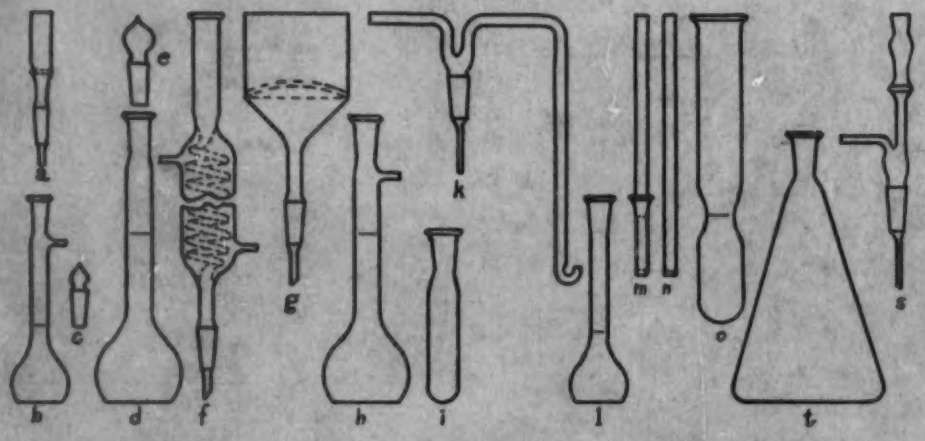

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